Long-range connectivity defines learning-induced intrinsic plasticity of prelimbic neurons

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La Connectivité sur de Longue Distance Détermine la Plasticité Intrinsèque des Neurones Prélimbiques Induite par l'Apprentissage

Sous la direction de : Andreas FRICK

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Long-Range Connectivity Defines Learning-Induced Intrinsic Plasticity of Prelimbic Neurons

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Résumé

Le cortex préfrontal médian (mPFC) est nécessaire pour la formation des représentations contextuelles et l'expression de la mémoire suite au conditionnement de peur. Des études récentes ont montré des changements dépendants de l'apprentissage dans l'excitabilité intrinsèque des neurones du mPFC. Il n'est cependant pas établit, si ces changements se font à l'échelle régionale ou s'ils sont spécifiques du type neuronal. La connectivité spécifique et les propriétés intrinsèques de différents types neuronaux pourraient entrainer certaines populations neuronales à être préférentiellement impliquées dans le traitement de l'information au cours d'une tâche d'apprentissage. Dans ce projet, nous avons étudié cette hypothèse par l'étude de la plasticité de l'excitabilité intrinsèque dans la partie prélimbique (PL) du mPFC dans deux groupes neuronaux bien définis : ceux projetant vers l'amygdale ipsilatérale et ceux projetant vers le mPFC controlatéral. Nous avons utilisé à la fois le conditionnement à la peur contextuelle, un traçage rétrograde, et des enregistrements électrophysiologiques en cellule entière des neurones pyramidaux marqués chez les souris mâles C57bl/6J adultes âgées de 2 à 3 mois. Nous montrons que l'excitabilité des neurones projetant vers l'amygdale présentent des changements dépendants de l'apprentissage, suite au conditionnement de peur contextuelle. En revanche, l'excitabilité des neurones projetant vers le mPFC controlatéral ne présente pas de différence entre les animaux entrainés et témoins. Ensemble, ces résultats indiquent que les changements induits par l'apprentissage dans l'excitabilité intrinsèque ne sont pas généralisés à tous les neurones du PL mais sont par contre définis par les cibles des neurones qui projettent sur de longues distances.

Mots-clés: *Cortex préfrontal médian*, *cortex prélimbique*, *conditionnement de peur contextuelle, excitabilité intrinsèque, apprentissage et mémoire*

Abstract

The medial prefrontal cortex (mPFC) is necessary for the formation of contextual representations and memory expression following fear conditioning. Recent studies have shown learning-dependent changes in the intrinsic excitability of mPFC neurons. It is not clear, however, whether these changes are region-wide or neuron-type specific. The specific connectivity and intrinsic properties of different neuronal types could cause certain neuronal populations to be preferentially involved in information processing in a learning paradigm. In this project, we investigated this hypothesis by studying the plasticity of intrinsic excitability in the prelimbic (PL) part of the mPFC in two defined neuronal groups: those projecting to the ipsilateral amygdala and those projecting to the contralateral mPFC. We used contextual fear conditioning together with retrograde tracing and whole-cell electrophysiological recordings of labelled pyramidal neurons in adult 2-3 month old male C56BL/6J mice. We show that neurons projecting to the amygdala display learning-dependent changes in neuronal excitability following contextual fear conditioning. In contrast, the excitability of neurons projecting to the contralateral mPFC does not differ between trained and control animals. Together, these results indicate that learning-induced changes in intrinsic excitability are not generalised across all PL neurons but instead are defined by the neurons' long-range projection targets.

Keywords: *Medial prefrontal cortex, prelimbic cortex, contextual fear conditioning, intrinsic excitability, learning and memory*

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List of Abbreviations

AB	Accessory basal nucleus of the amygdala
AC	Anterior cingulate cortex
ACd/Cg1	Anterior cingulate cortex, dorsal
aCSF	Artificial cerebrospinal fluid
ACv/Cg2	Anterior cingulate cortex, ventral
AGm	Medial agranular cortex
AHP	Afterhyperpolarisation
AIS	Axon initial segment
AP	Action potential
APV	2-Amino-5-phosphonovaleric acid
Arch	Archaerhodopsin
BA	Basal nuclei of the amygdala
Bi	Basal nucleus of the amygdala, intermediate subdivision
BK	Big conductance Ca^{2+} activated K^{+} channels
BLA	Basolateral amygdala
BMmc	Basomedial nucleus of the amygdala, magnocellular subdivision
BMpc	Basomedial nucleus of the amygdala, parvocellular subdivision
BOT	Bed nucleus of the olfactory tract
Bpc	Basal nucleus of the amygdala, parvocellular subdivision
CA1, CA2, CA3	Cornu ammonis fields 1-3
CaMKII	Ca^{2+}/calmodulin – dependant protein kinase
cAMP	Cyclic adenosine monophosphate
Cav	Voltage-gated Ca^{2+} channels
CB	Calbindin
CC	Corpus callosum
CCK	Cholecytokinin
CEA/CeA	Central nucleus of the amygdala
CEc	Central nucleus of the amygdala, capsular subdivision
CEl	Central nucleus of the amygdala, lateral subdivision
CEm	Central nucleus of the amygdala, medial subdivision
ChR2	Channelrhodopsin-2
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
COa	Cortical nucleus of the amygdala, anterior subdivision
COND	Conditioned mice
COp	Cortical nucleus of the amygdala, posterior subdivision
CAL	Calretinin
CR	Conditioned response
CREB	cyclic adenosine 3´,5´-monophosphate response element binding protein
CS	Conditioned stimulus
CtB	Cholera toxin B
CTX	Context only mice
DAB	3,3'-Diaminobenzidine tetrahydrochloride
DG	Dentate gyrus
DL-AP5	DL-2-Amino-5-phosphonopentanoic acid sodium salt

EC	Entorhinal cortex
EPSP	Excitatory postsynaptic potentials
fAHP	Fast afterhyperpolerisation
FR2	Frontal area 2
GABA	γ-aminobutyric acid
HCN	Hyperpolarisation activated and cyclic nucleotide-gated channels
HF	Hippocampal formation
IL	Infralimbic cortex
IMM	Immediate shock mice
IPSP	Inhibitory postsynaptic potentials
ISI	Interspike interval
ITC/In	Intercalated cell masses/Intercalated nuclei
K_{2P}	Two-pore K^+ channels
Kir3/GIRK	G protein–coupled inwardly–rectifying potassium channel
Kv	Voltage-gated K^+ channels
LA	Lateral nucleus of the amygdala
Ld	Lateral nucleus of the amygdala, dorsal subdivision
LTD	Long-term depression
LTP	Long-term potentiation
Lvl	Lateral nucleus of the amygdala, ventrolateral subdivision
Lvm	Lateral nucleus of the amygdala, ventromedial subdivision
M	Medial nucleus of the amygdala
M2	Secondary motor area 2
mAHP	Medium afterhyperpolerisation
MAPK	Mitogen–activated protein kinase
Md	Medial nucleus of the amygdala, dorsal subdivision
MD	Mediodorsal nucleus of the thalamus
MO	Medial orbital cortex
mPFC	Medial prefrontal cortex
Mv	Medial nucleus of the amygdala, ventral subdivision
Nav	Voltage-gated Na^+ channels
NBQX	2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide disodium salt
NMDA	N-methyl-D-aspartate
NOS	Nitric oxide synthase
NPY	Neuropeptide Y
NS309	SK channel activator
OB	Olfactory bulb
PAG	Periaqueductal gray
PB	Phosphate buffer
PFA	Paraformaldehyde
Pir	Piriform cortex
PKA	Protein kinase A
PKC	Protein kinase C
PL	Prelimbic cortex

PrCm	Medial precentral cortex
PV	Parvalbumin
RE	Nucleus reuniens of the midline thalamus
RMP	Resting membrane potential
S	Subiculum
sAHP	Slow afterhyperpolerisation
SK	Small conductance Ca^{2+} activated K^{+} channels
SOM	Somatostatin
STDP	Spike-timing dependent plasticity
TTX	Tetrodotoxin
US	Unconditioned stimulus
VAB	Ventral agranular bundle
vHPC	Ventral hippocampus
VIP	Vasoactive intestinal peptide
VTA	Ventral tegmental area
XE-991	Kv7 channel blocker
ZAP	Impedance amplitude profile

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Introduction (française)

Une des caractéristiques les plus remarquables du cerveau est sa capacité à acquérir de nouvelles informations et les stocker pour produire des changements adaptatifs du comportement. Il s'agit donc d'un objectif neuroscientifique majeur, depuis plusieurs décennies, que de comprendre les mécanismes neurobiologiques permettant de coder, stocker et récupérer de façon fiable des informations. L'apprentissage et la mémoire sont généralement étudiés dans le contexte des changements de la force synaptique liés à l'activité, autrement dit à la plasticité synaptique. Cependant, il est maintenant clair que l'apprentissage et les différents patrons d'activité neuronale peuvent également induire des formes non-synaptiques diverses et généralisées de plasticité, telles que les changements dans l'excitabilité intrinsèque d'un neurone (Zhang et Linden, 2003; Frick et Johnston, 2005; Disterhoft et Oh, 2006; Mozzachiodi et Byrne, 2010). La plasticité intrinsèque peut être définie comme un changement dans les propriétés électriques du neurone induit par un large éventail de canaux ioniques présents dans la membrane plasmique neuronale (Migliore et Shepherd, 2002; Magee, 2008; Nusser, 2012). Tout changement dans la distribution subcellulaire, densité, propriétés biophysiques, ou les niveaux d'activité de ces canaux modifiera l'excitabilité de la membrane neuronale (Hille, 2001; Frick et Johnston, 2005). La plasticité intrinsèque affecte une large gamme de fonctions neuronales, y compris la libération pré-synaptique des neurotransmetteurs, l'intégration des signaux synaptiques, la génération de potentiels d'action ou le reflux actif de signaux électriques dans les dendrites. Fonctionnellement, il a été suggéré que la plasticité intrinsèque peux servir comme un déclencheur de la plasticité synaptique subséquente, de l'allocation et consolidation de la mémoire, ou même agir comme une partie de l'engramme lui-même (Zhang et Linden, 2003; Frick et Johnston, 2005; Disterhoft et Oh, 2006; Zhou et al., 2009; Mozzachiodi et Byrne, 2010; Benito et Barco, 2010; Szlapczynska et al., 2014).

Le cortex préfrontal médian (mPFC) est une région pivot pour les fonctions exécutives telles que la prise de décision, l'action orienté vers un but, la détection d'erreur et la mémorisation (Brown et Bowman, 2002; Fuster 2008; Euston et al., 2012). En outre, grâce à ses liens avec les régions sous-corticales du cerveau impliquées dans le traitement des états émotionnels, il est dans une position stratégique pour induire un comportement adaptatif par l'emprise sur les réactions

émotionnelles (Euston et al., 2012). Le mPFC est aussi parfaitement adapté à la formation d'associations durables entre les indices menaçants et le cadre environnemental. Il intègre des signaux monosynaptiques de l'hippocampe ventral (Thierry et al., 2000), ce qui est important pour l'acquisition d'informations contextuelles, et il est connecté de façon bidirectionnelle avec l'amygdale baso-latérale (BLA, McDonald, 1991, 1996; Vertes, 2004; Likhtik et al., 2005), le centre de l'apprentissage émotionnel (LeDoux , 2003). En effet, il est maintenant bien établi que le mPFC joue un rôle essentiel dans l'acquisition, la consolidation et le stockage des souvenirs de peur (Sacchetti et al., 2003; Frankland et al., 2004; Laviolette et al., 2005; Laviolette et Grace, 2006; Einarsson et Nader, 2012) ainsi que dans la médiation de leur expression et leur extinction (Quirk et Mueller, 2008; Burgos-Robles et al., 2009; Knapska et Maren, 2009; Herry et al., 2010; Sierra-Mercado et al., 2011).

Le conditionnement de la peur a émergé comme un modèle utile pour l'étude de l'apprentissage et de la mémoire. Sur l'ensemble des émotions, la peur est celle qui est la mieux comprise à la fois sur le plan comportemental et neurobiologique, ce qui permet une analyse détaillée de la relation entre le comportement et une activité neuronale spécifique. De plus, l'apprentissage de la peur chez les rongeurs est rapide et robuste, une seule session d'entrainement étant suffisante pour produire des souvenirs à vie (Fanselow, 1990; Anagnostaras et al., 2000; Gale et al., 2004). Cela en fait un modèle très utile pour l'étude de la mémoire avec une haute résolution temporelle (Frankland et al., 2006; Reijmers et al., 2007; Tayler et al., 2013).

Malgré une bonne compréhension des circuits du cerveau impliqués dans le traitement de la peur, les mécanismes cellulaires précis permettant l'acquisition, le stockage, et l'expression de la mémoire émotionnelle dans le mPFC sont encore mal définis. Une étude récente a montré que le conditionnement de la peur entraine une diminution de l'excitabilité intrinsèque des neurones corticaux infralimbiques (IL), alors que pour les mêmes neurones, l'apprentissage de l'extinction de la peur provoque une augmentation de l'excitabilité intrinsèque (Santini et al., 2008). Le IL est une région du mPFC impliqué dans l'extinction de la peur et il est incertain si de tels changements dans l'excitabilité se produisent également dans le PL - une région adjacente au mPFC impliqué dans l'acquisition et l'expression de la peur (Vidal-Gonzales et al., 2006; Corcoran et Quirk, 2007; Lauzon et al., 2009; Laurent et Westbrook, 2009; Sierra-Mercado et al., 2011). En outre, il n'est pas clair si les

changements d'excitabilité observés dans les neurones IL se font à l'échelle régionale ou sont spécifiques du type neuronal. Nous savons maintenant que le mPFC contient une population hétérogène de neurones dont les propriétés intrinsèques et les réponses à la neuro-modulation diffèrent en fonction des cibles des neurones qui projettent sur de longues distances (Dembrow et al., 2010).

Pour les raisons susmentionnées, nous avons formulé l'objectif de recherche suivant:

Objectif 1 – Pour déterminer si le conditionnement de peur induit des changements dans l'excitabilité intrinsèque de deux groupes neuronaux du PL: ceux qui projettent vers la BLA- et ceux qui projettent vers le mPFC.

Afin de répondre à cet objectif nous avons infusé le traceur rétrograde de la toxine cholérique B (CTB) dans la BLA ipsilatérale et/ou dans le mPFC controlatéral de souris C57BL/6J agé de 2-3 mois. Nous avons choisi de mettre l'accent sur le groupe du mPFC parce qu'il présente une très forte connectivité inter-hémisphérique (Vertes, 2004; Hoover et Vertes, 2007) qui pourrait être importante pour l'induction de réponses à des situations stressantes (Lupinsky et al., 2010). Nous avons choisi le groupe de la BLA parce que la voie PL-BLA est considérée comme nécessaire pour l'acquisition et l'expression de la mémoire de peur (Burgos-Robles et al., 2009; Stevenson, 2011; Vouimba et Maroun, 2011) .

Une semaine après la chirurgie, les souris ont été entrainées à une tâche de conditionnement de la peur contextuelle. Les souris conditionnées à la peur ont exploré le contexte de conditionnement pendant deux minutes, après quoi elles ont reçu trois chocs électriques non signalés au niveau des pattes avec des intervalles d'une minute. Les souris contrôles, quant à elles, ont exploré le contexte sans recevoir de chocs (« contexte seulement »). Un à quatre jours après la tâche comportementale, les souris ont été sacrifiées pour des enregistrements électrophysiologiques. Les propriétés intrinsèques des neurones pyramidaux marqués du PL des couches 2/3 et 5 ont été mesurées sur des tranches aigues de cerveau avec la technique d'enregistrement de type patch-clamp en mode cellule entière. Les résultats obtenus ont été comparés entre les groups conditionnés et « contexte seulement ». Nous avons trouvé que le conditionnement à la peur contextuelle induisait une plasticité

intrinsèque dans les neurones du PL projetant vers la BLA mais pas dans les neurones projetant vers le mPFC.

Compte tenu de ces résultats, nous avons formulé notre second objectif de recherche :

Objectif 2 – Pour déterminer si les changements dans l'excitabilité intrinsèque observés dans les neurones du PL projetant vers la BLA étaient dépendants de l'apprentissage ou s'ils ont pu résulter de la douleur et/ou du stress associé avec le choc.

Afin de répondre à cet objectif, nous avons introduit un troisième groupe comportemental - le groupe de choc immédiat. Dans ce groupe, les souris ont reçu trois chocs non signalés aux pattes, tout comme les souris conditionnées, mais au lieu d'avoir eu le temps d'explorer d'abord le contexte, elles ont reçu des chocs dès leur placement dans la chambre de conditionnement. Dans un protocole de choc immédiat les animaux ne parviennent pas à développer une réponse conditionnée au contexte (Fanselow, 1986) et ce type de protocole est couramment utilisé comme un contrôle comportemental de l'effet délétère du choc électrique. Les propriétés intrinsèques des neurones pyramidaux marqués du PL des couches 2/3 et 5 provenant des souris ayant reçu un choc immédiat ont été mesurées seulement dans les neurones projetant vers la BLA. Les résultats ont été comparés à ceux obtenus avec les souris conditionnées et les souris du groupe « contexte seulement ».

Nos résultats ont montré que les changements induits par le conditionnement à la peur contextuelle dans les neurones PL projetant vers la BLA étaient significativement différents entre les souris conditionnées et ayant subi des chocs immédiats. Notamment, il n'y avait pas de différences significatives entre les propriétés intrinsèques des neurones issus des souris ayant subi des chocs immédiats et le « contexte seulement ». Par conséquent, en utilisant les souris ayant subi des chocs immédiats comme notre deuxième groupe de contrôle, nous avons établi que les changements dans l'excitabilité intrinsèque des neurones PL projetant vers la BLA ont été effectivement induits par l'apprentissage et n'ont pas été causés par l'expérience répulsive aux chocs électriques eux-mêmes.

En résumé, nos résultats indiquent que le conditionnement à la peur contextuelle induit des changements dépendant de l'apprentissage dans l'excitabilité

intrinsèque des neurones PL et ceci d'une manière spécifique à une population neuronale. Les populations de neurones qui subissent une plasticité intrinsèque peuvent être distinguées en fonction de leur connectivité sur de longue distance.

Ma contribution dans ce travail a été d'établir, exécuter et analyser toutes les expériences présentées dans cette thèse. J'ai également participé à la rédaction et à la révision du chapitre d'un livre référencé ci-dessous:

Szlapczynska, M.*, Bonnan, A.*, Ginger, M. and Frick, A. (2014). Plasticity and pathology of dendritic intrinsic excitability, In Horizons in Neuroscience Research, Vol. 14, A. Costa and E. Villalba, eds. (New York: Nova Science Publishers).
* Indique que le premier auteur est commun. J'ai écrit la section « Plasticité de l'excitabilité dendritique ». Le chapitre du livre est disponible en annexe.

1. Introduction

One of the most remarkable features of the brain is its ability to acquire new information and store it to produce adaptive changes in behaviour. It is therefore an ongoing neuroscientific aim to understand the neurobiological mechanisms allowing us to encode, store and reliably retrieve information. Learning and memory are typically studied in the context of activity-dependent changes in synaptic strength, namely synaptic plasticity. However, it is now clear that learning and different patterns of neuronal activity can also induce diverse and widespread non-synaptic forms of plasticity such as changes in the intrinsic excitability of a neuron (Zhang and Linden, 2003; Frick and Johnston, 2005; Disterhoft and Oh, 2006; Mozzachiodi and Byrne, 2010). Intrinsic plasticity can be defined as a change in the neuron's electrical properties mediated by a wide range of ion channels present in the neuronal membrane (Migliore and Shepherd, 2002; Magee, 2008; Nusser, 2012). Any change in the subcellular distribution, density, biophysical properties, or activity levels of these channels will alter the excitability of the neuronal membrane (Hille, 2001; Frick and Johnston, 2005). Intrinsic plasticity affects a wide range of neuronal functions including the presynaptic release of neurotransmitters, the integration of synaptic inputs, the generation of action potentials and the active back-flow of information into the dendrites. Functionally, it has been suggested to serve as a trigger for subsequent synaptic plasticity, memory allocation and consolidation, or even act as part of the engram itself (Zhang and Linden, 2003; Frick and Johnston, 2005; Disterhoft and Oh, 2006; Zhou et al., 2009; Mozzachiodi and Byrne, 2010; Benito and Barco, 2010; Szlapczynska et al., 2014)

The medial prefrontal cortex (mPFC) is a pivotal region for executive function such as decision-making, goal-oriented action, error detection and memory (Brown and Bowman, 2002; Fuster 2008; Euston et al., 2012). Moreover, thanks to its connections with subcortical brain regions implicated in the processing of emotional states, it is in a strategic position to mediate adaptive behaviour through the control over emotional responses (Euston et al., 2012). The mPFC is also perfectly suited for the formation of long lasting associations between threatening cues and environmental contexts. It integrates monosynaptic inputs from the ventral hippocampus (Thierry et al., 2000), which is important for the acquisition of contextual information, and it is bidirectionally connected to the basolateral amygdala (BLA; McDonald, 1991, 1996; Vertes, 2004; Likhtik et al., 2005), the centre for emotional learning (LeDoux, 2003). Indeed, it is now well established that the mPFC plays a critical role in the acquisition, consolidation and storage of fear memories (Sacchetti et al., 2003; Frankland et

al., 2004; Laviolette et al., 2005; Laviolette and Grace, 2006; Einarsson and Nader, 2012) as well as in mediating their expression and extinction (Quirk and Mueller, 2008; Burgos-Robles et al., 2009; Knapska and Maren, 2009; Herry et al., 2010; Sierra-Mercado et al., 2011).

Fear conditioning has emerged as a useful model for the study of learning and memory. Out of all the emotions, fear is the one that is best understood both on the behavioural and neurobiological level, which allows for a detailed analysis of the relationship between behaviour and specific neuronal activity. Moreover, fear learning in rodents is rapid and robust with a single training session being sufficient to produce life-long memories (Fanselow, 1990; Anagnostaras et al., 2000; Gale et al., 2004). This makes it a very useful model for the study of memory with a high temporal resolution (Frankland et al., 2006; Reijmers et al., 2007; Tayler et al., 2013).

Despite a good understanding of the brain circuits involved in fear processing, the precise cellular mechanisms underlying emotional memory acquisition, storage and expression in the mPFC are, however, still poorly defined. A recent study has shown that fear conditioning causes a decrease in the intrinsic excitability of infralimbic cortical (IL) neurons, whereas for the same neurons fear extinction learning causes an increase in intrinsic excitability (Santini et al., 2008). IL, is an mPFC region implicated in fear extinction and it is unclear whether such changes in excitability also occur in the PL - a neighbouring mPFC region implicated in fear acquisition and expression (Vidal-Gonzales et al., 2006; Corcoran and Quirk, 2007; Lauzon et al., 2009; Laurent and Westbrook, 2009; Sierra-Mercado et al., 2011). Additionally, it is not clear, whether the excitability changes observed in the IL neurons are region-wide or neuron-type specific. It is now known that the mPFC contains a heterogenous population of neurons whose intrinsic properties and responses to neuromodulation differ depending on the neurons' long-range projection targets (Dembrow et al., 2010).

Given the above reasons, we formulated the following research objective:

Objective 1 – To investigate whether fear conditioning induces changes in the intrinsic excitability of two neuronal groups in the PL: the BLA- and the mPFC-projecting neurons.

In order to address this objective we infused the retrograde tracer Cholera Toxin B (CtB) into the ipsilateral BLA and/or into the contralateral mPFC of 2-3 month old C57BL/6J mice. We

chose to focus on the mPFC-projecting group because the mPFC exhibits very strong interhemispheric connectivity (Vertes, 2004; Hoover and Vertes, 2007) that might be important for mediating responses to stressful situations (Lupinsky et al., 2010). We selected the BLA-projecting group because the PL-BLA pathway is thought to mediate fear memory acquisition and expression (Burgos-Robles et al., 2009; Stevenson, 2011; Vouimba and Maroun, 2011).

One week following surgery the mice were trained in a contextual fear conditioning task. The fear conditioned mice explored the conditioning context for two minutes after which they received three unsignalled footshocks at one minute intervals. On the other hand, the control mice (context only) explored the context without receiving any footshocks. One to four days after the behavioural task, the mice were sacrificed for electrophysiological recordings. The intrinsic properties of layer 2/3 and 5 labelled pyramidal PL neurons were measured in acute brain slices using somatic whole-cell current clamp recordings. The obtained results were compared between the conditioned and context only groups. We found that contextual fear conditioning induced intrinsic plasticity in the BLA-projecting PL neurons but not in the mPFC-projecting neurons.

Given those results we formulated our second research objective:

Objective 2 – To investigate whether the changes in intrinsic excitability observed in the BLA-projecting PL neurons were learning-dependent or whether they could have resulted from the pain and/or stress associated with the footshock.

In order to address this goal we introduced a third behavioural group – the immediate shock group. In this group, the mice received three unsignalled footshocks, just like the conditioned mice did, but instead of being given time to initially explore the context they received the shocks immediately upon placement in the conditioning chamber. In an immediate shock protocol the animals fail to develop a conditioned response to the context (Fanselow, 1986) and this type of protocol is commonly used as a behavioural control for the aversive effect of the footshock. The intrinsic properties of layer 2/3 and 5 labelled pyramidal PL neurons from the immediate shock mice were measured only in the BLA-projecting neurons. The results were compared to those obtained from the conditioned and context only mice.

Our results showed that the changes induced by contextual fear conditioning in the BLA-projecting PL neurons were significantly different between conditioned and immediate shock mice. Importantly, there were no significant differences between the intrinsic properties of neurons from the immediate shock and context only mice. Therefore, by using the immediate shock as our second control group, we established that the changes in the intrinsic excitability of the BLA-projecting PL neurons were indeed induced by learning and were not caused by the aversive experience of the footshock itself.

In summary, our results indicate that contextual fear conditioning induces learning-dependent changes in the intrinsic excitability of PL neurons and it does so in a neuronal population specific manner. The neuronal populations that undergo intrinsic plasticity can be distinguished based on their long-range connectivity.

My contribution to this work was to establish, perform and analyse all of the experiments presented in this thesis. I also participated in the writing and revision of the following book chapter:

Szlapczynska, M.*, Bonnan, A.*, Ginger, M. and Frick, A. (2014). Plasticity and pathology of dendritic intrinsic excitability, In Horizons in Neuroscience Research, Vol. 14, A. Costa and E. Villalba, eds. (New York: Nova Science Publishers).
* indicates shared first authorship. I wrote the section 'Plasticity of dendritic excitability'. The book chapter is attached in the Appendix.

2. The Neurobiology of Fear Conditioning

In Chapter 2 I describe Pavlovian fear conditioning as a model for studying neuronal mechanisms involved in learning and memory. I also discuss the neural circuits and mechanisms involved in the processing of fearful stimuli, with a special focus on the role played by the hippocampus and the amygdala. The role played by the medial prefrontal cortex (mPFC) in fear conditioning is explored in Chapter 3 – The Medial Prefrontal Cortex**.** I start this section by providing a general background into memory systems.

2.1. Memory systems

Learning and memory are fundamental to human life allowing us to acquire new knowledge that can be used to plan into the future and respond adaptively to unfamiliar situations. In humans, memory can be divided into short-term and long-term memory (Baddeley, 2001). Short-term memory lasts on the scale of seconds to a few hours. These memories are readily lost and are very sensitive to disruption. Working memory, often called 'online memory' is a subtype of short-term memory that allows us to temporarily store and manipulate information. It requires attention, active rehearsal and it is limited in its storage capacity (Baddeley and Hitch, 1974). Long-term memories, on the other hand, are permanent with virtually unlimited storage capacity. They undergo a process of consolidation before they are available for reliable retrieval. Short-term memory is not within the scope of this project and therefore from this point onward I will focus only on long-term memory.

Long-term memory can be grossly divided into two types - declarative and nondeclarative memory (Figure 2.1). Declarative memory, also known as *explicit memory* is the memory for facts (semantic memory) and events (episodic memory, Schacter and Tulving, 1994). It is available for conscious recollection, depends on the medial temporal lobe structures and it is affected by amnesia. Declarative memory forms our representation of the outside world, it allows us to encode the relationship between multiple items and events, it is flexible and the stored material can guide our performance in a variety of situations. It is also the kind of memory that we usually refer to when talking about memory (Squire, 1992). Non-declarative, or *implicit*, memory on the other hand is acquired through experience and can be readily accessed without our awareness when the circumstances require it. It is expressed through performance and does not require a conscious recollection of the past. The recall of these memories occurs through the reactivation of the systems through which the learning had occurred originally. Non-declarative memory can be further subdivided into i) procedural

memory - the memory for skills, habits, ii) priming and perceptual learning iii) non-associative learning - such as sensitisation and habituation, as well as iv) associative learning such as simple forms of classical conditioning (Squire, 1992, 2004). Despite this categorisation it is important to bear in mind that in most life situations multiple forms of memory are activated in parallel. For example, when stung by a bee we could form a declarative memory of the exact day and location where the unpleasant event had happened as well as a non-declarative memory, namely the fear of bees.

Long-term memory is not a unitary system and no one brain area or cell type can account for all of the memories we store. Nevertheless, it is now clear that certain brain areas are critical for some types of learning to occur. For example, spatial memory is known to require the hippocampus (O'Keefe and Nadel, 1978), whereas emotional learning (e.g. fear conditioning) depends primarily on the amygdala (LeDoux, 2003).

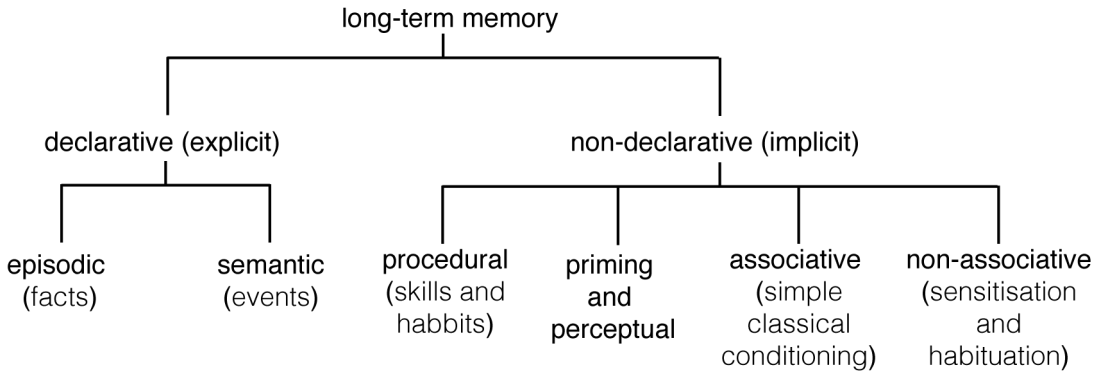


Figure 2.1 Long-term memory systems. The accepted division of memories into declarative and nondeclarative memories as well as their individual subtypes. Adapted from Squire et al., 2004.

2.2 Pavlovian fear conditioning

Fear conditioning is a form of classical conditioning typically classified as a nondeclarative associative form of learning and memory (for fear conditioning as a model for declarative memory see section 2.3.1 *Hippocampus in fear conditioning*). Arguably some of the most famous studies of classical conditioning date back to the 1920s. In 1927 Ivan Pavlov demonstrated that when he rang a bell just before he gave food to a group of dogs, over time the dogs started salivating to the sound of the bell (Pavlov, 1927). This important observation showed that the dogs formed an association between a conditioned stimulus (CS) - the sound of a bell - and the occurrence of an unconditioned stimulus (US) - the food. The salivating

that was originally a response only to the US became, through the process of association, a conditioned response (CR) to the CS. Another study, performed by John Watson and Rosalie Rayner (1920), demonstrated a famous case of fear conditioning, in what is known as the 'Little Albert' experiment. Before the start of the experiment, little Albert an 11-month-old infant showed no fear towards a white rat that he was curious of and happily played with. However, from the onset of the experiment, every time little Albert would extend his hand towards the rat the experimenters banged a steel bar with a hammer behind the infant's head. This sudden noise caused little Albert to startle violently and brake into sudden crying. After several pairings of the rat and the noise, little Albert started displaying a conditioned fear of the rat. When presented with the rat he would start crying and try to move away from it (Watson and Rayner, 1920).

Nowadays, fear conditioning has become a useful and widely used paradigm for studying emotional learning and memory. In a typical fear conditioning paradigm a mouse or a rat is placed in a conditioning chamber and following several minutes of free exploration it is exposed to a neutral sensory stimulus such as a tone, light or odour, which is then quickly followed by an aversive event such as a brief electric footshock. After repeated presentations of the stimulus-shock pairings the animal develops a conditioned fear of the stimulus. Following the acquisition of the CS-US association, the innate physiological and behavioural responses begin to be controlled by the CS. Defensive responses that are typical for rodents include cessation of movement (freezing), autonomic arousal (increase in heart-rate, blood pressure, respiration), an endocrine response (increase in stress hormone release), alterations in pain sensitivity (hypoalgesia), ultrasonic vocalisations and enhanced reflex expression such as fear-potentiated startle and eyeblink responses (LeDoux, 2000; Kim and Jung, 2006).

The behavioural measure most commonly used to assess conditioned fear memory in the laboratory, is freezing. Freezing can be defined as a complete lack of movement apart from breathing (Fanselow, 2000). Although perhaps not the most accurate index of actual fear, it is widely used across laboratories. This is because it does not require any additional equipment, as would be necessary for measuring the heart rate or ultrasonic vocalisations, it is clearly defined, readily observable and easily measured, allowing for results from different laboratories to be easily compared.

2.2.1 Contextual fear conditioning

The most commonly studied type of fear conditioning is cued fear conditioning, where the presentation of a discrete CS (usually a tone) signals the eminent occurrence of the US (shock). However, mice/rats also readily acquire conditioned fear of the context in which they experience the aversive stimulus. In a typical contextual fear conditioning training session the rodent is placed in the conditioning chamber and is allowed a few minutes of free exploration (usually 1-2 minutes) before it receives one or more unsignalled footshocks. Contextual fear can also be acquired during cued conditioning provided that enough time is allowed for exploration before the onset of the tone-shock pairing. In contextual fear conditioning the context, which can be vaguely defined as 'the set of circumstances around an event' (Maren et al., 2013), becomes the CS. Consequently, as opposed to cued fear conditioning the CS is: i) multisensory as it is composed of many different elements - the box where the animal is placed, the metal grid through which the shock is delivered, the presence of light, the odour, etc. ii) continuously present throughout the training session and iii) diffuse – it is predictive of the general situation in which the aversive stimulus will occur but is not predictive of the exact onset of this stimulus (Maren et al., 2013). Given the above, learning in contextual fear conditioning occurs under slightly different rules than in does in cued conditioning. Firstly, learning occurs through a process of exploration during which the animal assembles a configural representation of the context from all of its individual elements (Rudy and O'Reilly, 2001; Rudy et al., 2004; Maren et al., 2013). The final representation is not simply a sum of all the elements. Instead, the animal forms a new 'gestalt' representation of the context and it is this global representation that enters into an association with the US. During contextual fear conditioning we can therefore distinguish two distinct phases - context encoding and context conditioning, with context encoding being necessary for context conditioning to take place (Figure 2.2; Fanselow, 2010; Maren, 2013). Secondly, contextual and cued fear conditioning differ in the time lapse that is necessary to occur between the presentation of the CS and the US. In cued fear conditioning, the shorter the time interval between the CS and US presentation, the better the conditioning will be. In contrast, in contextual fear conditioning, learning is better when the delay between the placement of the animal in the conditioning chamber and the shock is extended (Fanselow, 2000).

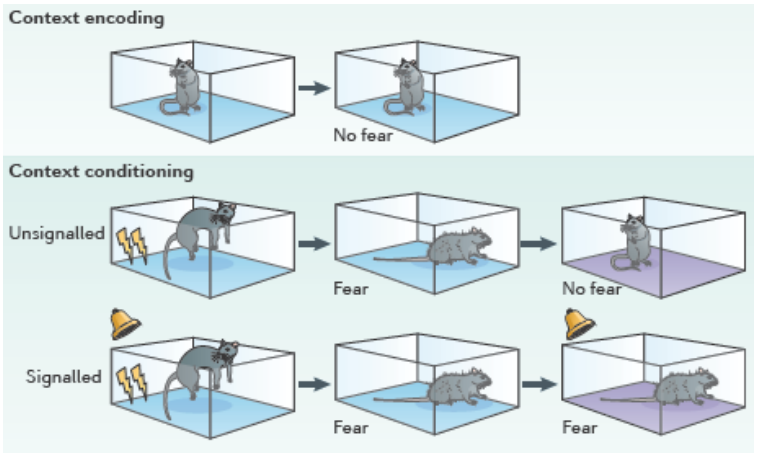


Figure 2.2 Context encoding and conditioning in rodents. When the rodent is exposed to a new context it automatically acquires its contextual representation in a process called context encoding (top panel). If that context is then paired with an aversive stimulus such an electric footshock (lightening symbol) it will result in the formation of an association between the context and the shock. This process is called context conditioning and it can occur for both unsignalled and signalled shocks (bottom panel). A shock is signalled when it is preceded by a distinct cue such a specific tone (bell symbol). Adapted from Maren et al., 2013.

The importance of this delay is clearly demonstrated by a phenomenon called the immediate shock deficit (Fanselow, 1986). If instead of being given time to explore the context, the rat is shocked immediately upon placement in the conditioning chamber it does not develop a CR to the context (Fanselow, 1986). A delay of approximately 20 seconds is necessary for any conditioning to occur (Fanselow, 1990, 2000; Landeira-Fernandez et al., 2006). This failure in conditioning might stem from insufficient contextual encoding and/or an inability of the animal to form a proper CS-US association. The immediate shock paradigm is therefore a useful behavioural control for the effect of the footshock. It allows for the comparison of the neurobiological responses of the animals that successfully underwent conditioning to those that did not even though both groups had been exposed to exactly the same aversive stimulus.

Despite the differences in the rules governing information processing in cued and contextual fear conditioning, both behavioural paradigms produce long-term memories following a single trial and result in identical emotional responses – conditioned freezing to the CS.

2.2.2 Conditioned fear as a model for studying memory

Fear is a defensive mechanism that we acquired through evolution to enable us to protect ourselves from danger and survive. It can be defined as an emotional state coupled with a behavioural and physiological response to a threating environment. Despite the fact that in

humans fear is very much a psychological construct with no single definition or interpretation, it is nonetheless an emotion that is not unique to us. It is conserved across all non-human mammals as well as probably most vertebrates. The study of fear is important because it plays a major role in our lives and can be the basis of many psychopathological conditions, such as anxiety disorders, phobias, panic or posttraumatic stress disorder (Davis, 1992; Maren and Quirk, 2004; Phelps and LeDoux, 2005; Pape and Paré, 2010).

Fear conditioning is a very good model for the study of learning and memory. Fear is the emotion that is best understood behaviourally and in terms of the brain circuits involved. This allows for a detailed analysis of the relationship between behaviour and neuronal activity. Moreover, fear learning is very robust in mice and rats, fear induction is rapid and often a single training trial is enough to produce life-long memories (Fanselow, 1990; Anagnostaras et al., 2000; Gale et al., 2004). This is useful for the study of different memory phases with high temporal resolution (Frankland et al., 2006; Reijmers et al., 2007; Tayler et al., 2013). Most importantly however, fear is a valuable tool for neuroscientific research because it is induced by clear and specific environmental cues that are under the experimenter's control. These stimuli evoke measurable effects in the form of distinct behavioural and physiological responses (LeDoux, 2000; Kim and Jung, 2006). Finally, studying fear memory is not labour intensive - a typical training session last approximately 5-10 min and the equipment is compact allowing for many mice to be tested at once.

2.3 The neuronal circuit of fear

Just as with any kind of memory, the acquisition and storage of fear memories is not restricted to a single brain structure and instead requires the interaction of neuronal circuits located within many different regions. There is a general consensus that the three main structures mediating the encoding, storage and retrieval of contextual fear memories are the amygdala, hippocampus and the medial prefrontal cortex (Figure 2.3; mPFC; Tronson et al., 2012; Maren et al., 2013; Zelikowsky et al., 2013).

It is now well established that contextual representations are formed within the hippocampo-cortical networks, whereas the amygdala is the place where the CS-US associations are made and whose output directly elicits conditioned freezing (Sah et al., 2003; Kim and Jung, 2006; Pape and Paré, 2010). The mPFC is known to be the site for remote fear memory storage (Frankland et al., 2004), as well as for mediating fear expression (Burgos-

Robles et al., 2009; Sierra-Mercado et al., 2011), extinction (Quirk and Mueller, 2008; Herry et al., 2010), and the renewal of extinguished fear responses (Knapska and Maren, 2009).

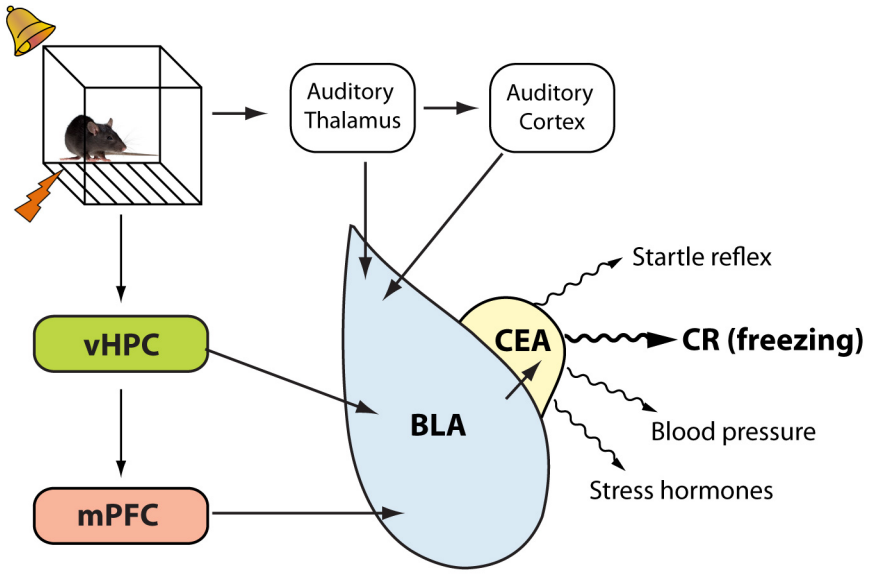


Figure 2.3 The neuronal circuit of fear. Abbreviations: BLA, basolateral amygdala; CEA, central nucleus of the amygdala; CR, conditioned response; mPFC, medial prefrontal cortex; vHPC, ventral hippocampus.

Below I discuss the role played by the hippocampus and the amygdala in conditioned fear learning and memory. The contribution of the mPFC is treated separately in Chapter 3, which is specifically dedicated to this structure.

2.3.1 Hippocampus

The rodent hippocampal formation (HF), commonly just referred to as the hippocampus, is a structure located in the medial temporal lobe. The HF is a collection of brain areas comprising the dentate gyrus (DG), the hippocampus proper, the subiculum, presubiculum, parasubiculum and the entorhinal cortex (Figure 2.4; Amaral and Lavenex, 2007). The hippocampus proper is further subdivided into the CA3, CA2 and CA1 (CA stands for *Cornu Ammonis*). Depending on the nomenclature, the presubiculum, parasubiculum and the entorhinal cortex can also be classified as forming part of the parahippocampal region and not being part of the HF itself (Amaral and Lavenex, 2007; van Strien et al., 2009). The anatomy of the HF differs along the septotemporal axis (Figure 2.4). The extreme septal division

contains only the CA3-CA1 fields and the DG. The subiculum is first visible about a third of the way along the axis, with the presubiculum and parasubiculum emerging towards the temporal division. The entorhinal cortex is located even more caudally and ventrally (Amaral and Lavenex, 2007). The entorhinal cortex is reciprocally connected with the neocortex and it gives rise to the perforant path that projects to other regions of the HF and thus constitutes the main cortical input pathway. The subiculum on the other hand is the main source of subcortical projections (Amaral and Lavenex, 2007; van Strien et al., 2009).

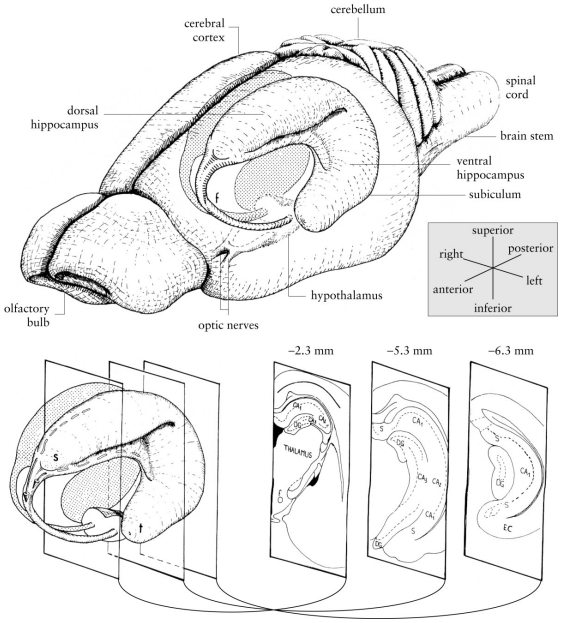


Figure 2.4 The three-dimensional organisation of the rodent hippocampal formation. Three coronal sections of the hippocampal formation showing the differences in anatomy along the septo-temporal axis. Abbreviations: CA1, CA2, CA3 - cornu ammonis fields 1–3; DG, dentate gyrus; EC, entorhinal cortex; f, fornix; s, septal pole of the hippocampus; S, subiculum; t, temporal pole of the hippocampus. Adapted from Amaral and Witter (1995) and Cheung and Cardinal (2005).

Hippocampus in fear conditioning

Following the discovery of place cells by John O'Keefe it became apparent that the rodent hippocampus does not simply register information about a single sensory modality such as visual, auditory or painful stimuli, but instead it assembles and stores a rich contextual representation of the space surrounding the animal (O'Keefe and Dostrovsky, 1971). This hippocampo-dependent spatial representation of a context also called a 'cognitive map' is what the animal uses to navigate its environment (O'Keefe and Nadel, 1978). These cognitive maps are acquired rapidly and automatically upon placement in the context and are a direct

consequence of the animal attending to its environment (Morris and Frey, 1997; Rudy and O'Reilly, 2001). Once this representation is formed it can be relayed to the amygdala where it is then associated with the US (Fanselow, 2000; Sanders et al., 2003).

Given the importance of the hippocampus in contextual encoding, its role in the formation of contextual fear memory seems evident. Indeed, contextual fear conditioning results in an increased expression of the immediate early genes *cfos* and *zif268* (Frankland et al., 2004). In behavioural training, the detection of *cfos* and *zif268* is often used to indicate which neurons were activated during learning. Contextual fear conditioning also results in structural changes in the hippocampus such as an increase in dendritic spine numbers (Restivo et al., 2009). Furthermore, the inactivation of the ventral hippocampus prior to memory retrieval with the $GABA_A$ (γ-aminobutyric acid) receptor agonist muscimol impairs both fear expression and extinction memory (Sierra-Mercado et al., 2011; Sotres-Bayon et al., 2012). However, the strongest evidence for the role of the hippocampus in encoding fear memory has been provided recently. Tonegawa and colleagues showed that the re-activation of DG neurons originally engaged by fear conditioning was sufficient to induce freezing in a group of mice. When these neurons were re-activated mice exhibited freezing responses to a context that they were familiar with but in which they had never received a footshock (Liu et al., 2012). This experiment provides direct evidence for the involvement of hippocampal cells in fear memory encoding and retrieval.

The role of the hippocampus is well established for declarative memory in humans. Damage to the hippocampus causes selective memory impairments in the form of both anterograde (the inability to form new memories) and retrograde amnesia (the loss of memories acquired prior to hippocampal damage). Retrograde amnesia usually follows a temporal gradient, where recently acquired memories are lost, while the ones from the more remote past are spared. Nondeclarative memories on the other hand remain intact in amnesic patients with medial temporal lobe damage (Squire, 1992). A significant body of evidence from animal studies of fear conditioning shows that rodents with hippocampal lesions display memory deficits similar to those observed in humans. Indeed, hippocampal lesion and inactivation studies cause both retrograde (Kim and Fanselow, 1992; Frankland et al., 1998; Anagnostaras et al., 1999; Flavell and Lee, 2012) and in certain cases anterograde amnesia of contextual fear memory (Phillips and LeDoux, 1992; Young et al., 1994; Maren et al., 1997). This retrograde amnesia follows a similar temporal gradient of information loss to that observed in humans (Bayley et al., 2005; Squire and Bayley, 2007). Indeed, when

hippocampal lesions are made shortly after training (one day) mice display deficits in the CR - freezing. On the other hand when the lesions are made at more remote time points (one month) following training, the CR appears to be preserved (Kim and Fanselow, 1992; Anagnostras et al., 1999). Importantly, the consequences of hippocampal lesions produce more selective memory deficits for contextual fear than they do for tone fear (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Anagnostaras et al., 1999; although some reports indicate that memory for tone is also impaired Maren and Fanselow, 1995; Maren et al., 1997). Because of this, contextual fear conditioning has received considerable attention as a model resembling that of declarative memory in humans (Squire, 1992; Fanselow, 2000; Rudy et al., 2002).

Despite the similarities, certain differences between human and animal studies do exist. While hippocampal lesions performed after contextual fear training consistently abolish freezing (Kim and Fanselow, 1992; Maren et al., 1997; Frankland et al., 1998; Anagnostaras et al., 1999; Flavell and Lee, 2012), when the damage occurs before training, the animals show a remarkable ability to overcome the hippocampal loss given sufficient training (Maren et al., 1997; Frankland et al., 1998; Cho et al., 1999; Rudy et al., 2002; Wiltgen et al., 2006). A possible explanation comes from the fact that extrahippocampal structures are also capable of acquiring and storing contextual representations (Fanselow, 2010). While the hippocampus is a fast learning system, the cortex uses a slower learning rate that focuses on extracting generalities and statistical regularities (McClelland et al., 1995). Indeed, damage to the hippocampus slows down learning but it does not prevent the animals from acquiring new spatial information (de Hoz et al., 2005; Wiltgen et al 2006; Maren et al., 2013). In agreement with this, when rats are given more time for contextual encoding, the effect of the hippocampal lesion is eliminated (Young et al., 1994). In contrast, when the time is reduced contextual fear memory is impaired (Wiltgen et al., 2006). However, even if the cortex is sufficient for supporting contextual encoding in the absence of the hippocampus, the hippocampus may still be necessary for storing rich and detailed contextual representations (Nadel et al., 2000; Hyman et al., 2012).

2.3.2 Amygdala

The amygdala is a structure buried deep in the medial temporal lobe that has been identified as a key region for the processing of aversive signals as well as fear acquisition, expression and extinction (Davis, 1992; LeDoux, 2000; Sah et al., 2003; Maren and Quirk, 2004;

McGaugh, 2004; Phelps and LeDoux, 2005; Kim and Jung, 2006; Pape and Paré, 2010; Marek et al., 2013). The amygdaloid complex consists of numerous distinct and structurally diverse subnuclei with extensive inter- and intranuclear connections (Pitkänen et al., 2000a, 2000b; Sah et al., 2003; Pape and Pare, 2010). These nuclei are commonly classified into three subdivisions (Figure 2.5). These are: i) the deep basolateral group (BLA) consisting of the lateral (LA) and basal (BA) nuclei ii) the more superficial cortical-like group consisting of the cortical nuclei, and the nucleus of the olfactory tract, and iii) the centromedial group divided into the medial (M) and central nuclei (CEA). The remaining nuclei that are not easily categorised are the intercalated cell masses (ITC also referred to as the intercalated nuclei, In) and the amygdalahippocampal area (Pitkänen et al., 2000a, 2000b; Sah et al., 2003; Knapska et al., 2007). The amygdalar regions that are particularly important for fear conditioning are the BLA, the CEA the ITC (Pitkänen et al., 1997; Maren, 2003; Pape and Paré, 2010). The involvement of the ITC will however not be discussed.

Anatomically, the amygdala is well suited for the control of fear memories. The BLA is the primary sensory input zone of the amygdala. It receives direct sensory inputs from a range of brain areas such as the thalamus, neocortex, and hippocampus, and it is here that the association between the CS and US takes place. The information is then relayed to the CEA - the main output structure of the amygdala that drives the expression of conditioned fear through its connections to various autonomic and somatomotor areas, such as: the bed nucleus of stria terminals (for stress hormone release), the brainstem (for freezing) or the hypothalamus (for sympathetic activation). These areas in turn generate a range of behavioural responses associated with the expression of fear (Sah et al., 2003; Kim and Jung, 2006; Pape and Paré, 2010; Figure 2.3).

The neuronal composition of the BLA is heterogenous and consists of both glutamatergic projection cells (80%) and GABAergic interneurons. In contrast, the CEA is a striatal-like structure and consists of predominantly GABAergic neurons (Pitkänen et al., 1997; Pape and Paré, 2010; Marek et al., 2013). The CEA can be further subdivided into the medial (CEm) as well as the lateral (CEl) divisons (Cassell et al., 1986).

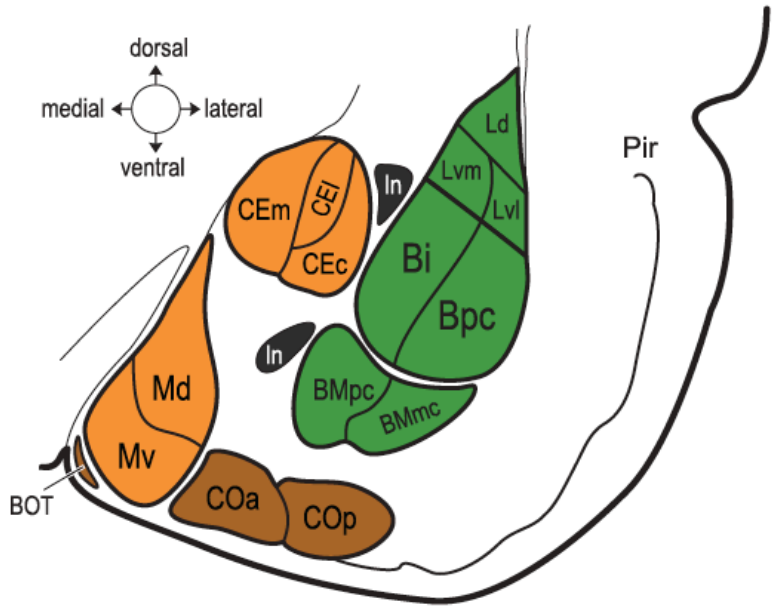


Figure 2.5 Anatomical subdivision of the amygdalar nuclei in the rat. The amygdaloid complex can be divided into three groups (see text): i) the deep basolateral group (green), ii) the more superficial cortical-like group (brown) and iii) the centromedial group (orange). The intercalated nuclei (In) are indicated in dark gray. Abbreviations: CEc, central nucleus, capsular subdivision; CEl, central nucleus, lateral subdivision; CEm, central nucleus, medial subdivision; COa, cortical nucleus, anterior subdivision; COp, cortical nucleus, posterior subdivision; BOT, bed nucleus of the olfactory tract; Bi, basal nucleus, intermediate subdivision; Bpc, basal nucleus, parvocellular subdivision; BMmc, basomedial nucleus, magnocellular subdivision; BMpc, basomedial nucleus, parvocellular subdivision; Ld, lateral nucleus, dorsal subdivision; Lvm, lateral nucleus, ventromedial subdivision; Lvl, lateral nucleus, ventrolateral subdivision; Md, medial nucleus, dorsal subdivision; Mv, medial nucleus, ventral subdivision; In, intercalated nuclei; Pir, piriform cortex. Taken from Knapska et al., 2007

Amygdala in fear conditioning

Early evidence for the role of the amygdala in fear learning comes from lesion studies. Kapp was one of the first to report the implication of the amygdala in fear memory by showing that lesions to the CEA abolished heart rate conditioning[1] in rabbits (Kapp, 1979). In another influential study from 1972, Blanchard and Blanchard showed that lesions to the amygdala impaired the expression of both innate and conditioned fear. Furthermore, the pharmacological inactivation of the BLA impairs conditioned freezing and memory extinction. It is now well established that amygdala lesions impair both the acquisition and the expression of conditioned fear (LeDoux et al., 1990a; Phillips and LeDoux, 1992; Kim et al., 1993; Maren and Fanselow, 1995; Maren et al., 1996a; Goosens and Maren, 2001). Support for the fact that the amygdala is the locus of conditioned fear also comes from electrophysiological studies. Using single unit recordings Herry and colleagues have

[1] Heart-rate conditioning can be used as an index to measure heart rate changes as a result of fear conditioning.

identified the existence of a specific population of so called 'fear neurons' in the BLA (Herry et al., 2008). These principal neurons increase in response to the CS that occurs during fear conditioning (Maren et al., 1991; Quirk et al., 1995; Goosens et al., 2003; Herry et al., 2008). Neurons responsive to the CS have also been shown to exist in the CEA (Applegate et al., 1982; Pascoe and Kapp, 1985).

The BLA is the main input structure of the amygdala. In auditory fear conditioning, sensory inputs arrive from the auditory thalamus and the auditory cortex and terminate in the LA (Romanski et al., 1993; Bordi and LeDoux, 1994). Both inputs are capable of eliciting responses to the CS (LeDoux et al., 1990a, Romanski and LeDoux, 1993; McDonald, 1998; LeDoux, 2000) and lesions of the LA or the auditory thalamus prevent the formation of tone-shock associations (LeDoux, 1986; LeDoux et al., 1990b). Contextual information reaches the BLA through hippocampal afferents. The ventral hippocampus (CA1 and subiculum) sends direct projections to the BA via the ventral agranular bundle (VAB, Canteras and Swanson, 1992). When these regions are damaged and/or the communication between them is impaired, there is a significant decrease in freezing to the conditioning context (Maren and Fanselow, 1995; Flavell and Lee, 2012). Interestingly, hippocampal inputs have been shown to directly target the CS responsive 'fear neurons' that have been identified in the BA (Herry et al., 2008). The BLA is therefore an important modulator of contextual fear memory. Indeed, muscimol infusions impair freezing to contextual stimuli, whereas the infusions of norepinephrine enhance it (Huff et al., 2005). However, even though the BLA is necessary for the formation of contextual fear memory it does not seem to play a role in the long-term cognitive/declarative aspect of this memory (Vazdarjanova and McGaugh, 1998).

As opposed to the BLA, the CEA has been identified as main output structure responsible for the generation of fear responses (LeDoux, 2000). Of particular interest are the intrinsic inhibitory networks within the CEA, which seem to shape the overall activity and output of this region (Ehrlich et al., 2009). Upon the presentation of the CS a subgroup of cells in the CEl called 'CEl-on neurons' become activated and in turn inhibit another tonically active CEl population called the 'CEl-off neurons'. Consequently, these 'off neurons' disinhibit neurons in the CEm, which in turn evokes a fear response (Ciocchi et al., 2010; Haubensak et al., 2010). The information flow between the BLA and the CEA is gated by the ITC (reviewed in Pape and Paré, 2010; Orsini and Maren, 2012).

The electrical stimulation of the amygdala in the absence of fear conditioning is sufficient to produce a range of behavioural and autonomic responses typical for the state of

fear. These include changes in heart rate, blood pressure and respiration. Moreover, the stimulation of the CEA in rodents results in freezing behaviour, whereas in humans the stimulation of the amygdala produces feelings of fear and anxiety. This suggests that, in fear conditioning, for a previously neutral stimulus to evoke a conditioned fear response it is only necessary for that stimulus to activate the amygdala. This, by the virtue of association will result in conditioned fear responses (Davis, 1992).

It is therefore not surprising that many groups are seeking to uncover long-term potentiation mechanisms (LTP) in the amygdala (for detailed reviews see Blair et al., 2001; Sigurdsson et al., 2007). Hebbian LTP is an associative form of plasticity where the repetitive excitation of the postsynaptic cell by the presynaptic cell results in the strengthening of the synaptic connection between them (Hebb, 1949). Hebbian plasticity is characterised by rapid induction and prolonged duration (Bliss and Lomo, 1973; Bliss and Collingridge, 1993). In a Hebbian model of fear conditioning, a strong depolarisation caused by the US results in the strengthening of the coactive CS inputs onto the same neurons (LeDoux, 2000; Blair et al., 2001; Paré, 2002; Sah and Westbrook, 2008; Johansen et al., 2011). Indeed, delivering high frequency stimulation to the auditory thalamic pathway results in LTP in the LA (Clugnet and LeDoux, 1990). Similarly, the VAB pathway carrying contextual information between the hippocampus and the basal amygdala (Canteras and Swanson, 1992) undergoes NMDA (N-methyl-D-aspartate) receptor dependent LTP. Furthermore, the infusion of NMDA receptor antagonists into the BLA prevents the acquisition of conditioned fear (Fanselow and Kim, 1994; Maren et al., 1996a, 1996b; Rodrigues et al., 2001).

Even though NMDA receptor dependent synaptic transmission may account for some of the plasticity occurring during fear conditioning in the amygdala, an alternate mechanism may also be involved. When the pre- and postsynaptic activity is paired during learning Ca^{2+} entry occurs through both NMDA receptors and voltage-gated Ca^{2+} channels. Therefore, the prevailing view now is that while NMDA receptor dependent plasticity is important for the acquisition of fear memory, voltage-gated Ca^{2+} channels are involved in its consolidation and long-term storage (Fanselow and Kim, 1994; Weisskopf et al., 1999; Rodrigues et al., 2001; Bauer et al., 2002; Shinnick-Gallagher et al., 2003; McKinney and Murphy, 2006).

3. The Medial Prefrontal Cortex

In Chapter 3 I review what is known about the role of the medial prefrontal cortex (mPFC) in fear conditioning. I start this section by describing the anatomy and connectivity of the mPFC. The role of the mPFC in other cognitive functions, especially long-term memory is also briefly reviewed. Special focus is given to the prelimbic (PL) part of the mPFC, this being the structure I have studied during my PhD.

3.1 Overview

The medial prefrontal cortex (mPFC) is known to play a role in a wide range of cognitive and executive tasks including attention, planning, goal-oriented action, decision-making, conflict monitoring, error detection as well as short and long-term memory (Miller, 2000; Miller and Cohen, 2001; Holroyd et al., 2002; Botvinick et al., 2004; Ridderinkhof et al., 2004; Euston et al., 2012). A large body of literature also supports the role of the mPFC in reward-guided learning based on risk/reward expectations (Bechara and Damasio, 2005; Rushworth et al., 2011) as well as drug seeking and addiction (Peters et al., 2009). Perhaps one useful umbrella term for incorporating all mPFC functions is its role in adaptive behaviour (Euston et al., 2012). Indeed, mPFC activity is strongly modulated by the subjective value of actual or anticipated outcomes (Rushworth et al., 2011), such as the subjective experience of pain (Johansen et al., 2001; Shackman et al., 2011) or the expectation of aversive events (Baeg et al., 2001; Gilmartin and McEchron, 2005). We study the mPFC because of its critical role in regulating emotional behaviour, including conditioned fear (Fuster, 2008; Courtin et al., 2013).

3.2 Anatomy and connectivity of the mPFC

In rodents, the mPFC can be subdivided into four distinct areas: the anterior cingulate cortex (AC, dorsal and ventral part, the equivalent of Brodmann's area 24), the prelimbic cortex (PL, area 32), the infralimbic cortex (IL, area 25, Krettek and Price, 1977; Ray and Price, 1992; Öngur and Price, 2000) and the medial precentral cortex (PrCm; Figure 3.1). Depending on the nomenclature the PrCm is also known as the medial agranular cortex (AGm) or the frontal area 2 (FR2, Krettek and Price, 1977; Uylings and Van Eden, 1990). However, Paxinos and Watson (2001) have adapted different nomenclature where the dorsal AC (ACd) and ventral AC (ACv) are called Cg1 and Cg2 respectively, whereas the PrCm is indicated as the secondary motor area (M2).

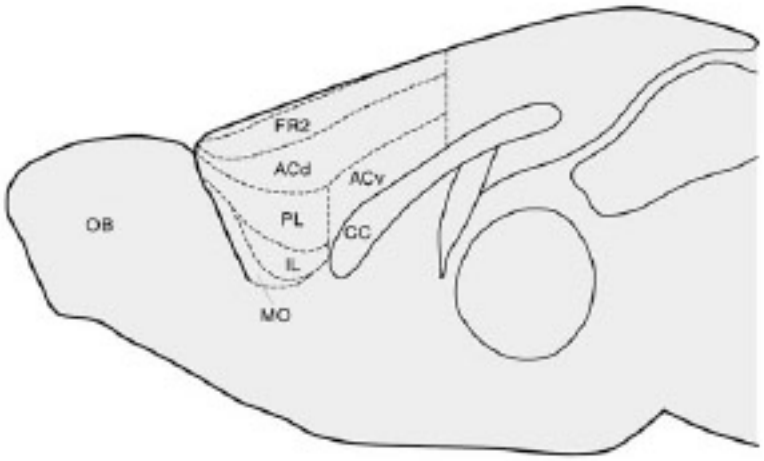


Figure 3.1 A schematic view of the mouse prefrontal cortex. Abbreviations: ACd, anterior cingulate cortex, dorsal part; ACv, anterior cingulated cortex, ventral part; CC, corpus callosum; FR2, frontal area 2; IL, infralimbic cortex; MO, medial orbital cortex; OB, olfactory bulb; PL, prelimbic cortex. Taken from van de Werd et al., 2010.

The mPFC is well connected with other subcortical areas to access and mediate emotional information processing. Indeed, it is reciprocally connected with the BLA, a critical region for emotional learning (LeDoux, 2003). It also communicates with the hypothalamus, which controls homeostatic states, such as hunger and thirst, as well as the autonomic and endocrine systems. It sends prominent connections to the dorso and ventromedial striatum, the periaqueductal gray (PAG) - a region implicated in aggression, defensive behaviour (also fear conditioning) and pain modulation – and to the lateral habenula, an area involved in learned responses to stress, anxiety, pain and reward. The mPFC is also reciprocally connected to a wide-range of neuromodulatory systems including the dorsal raphe, ventral tegmental area (VTA) and locus coeruleus. Finally, the mPFC receives direct inputs from the CA1 and the subiculum regions of the ventral hippocampus. Interestingly, no direct return projection exists from the mPFC to the hippocampus. It has been suggested that the nucleus reuniens (RE) of the midline thalamus acts like a relay structure in the transfer of information (Vertes, 2006; Vertes et al., 2007).

In terms of cortical connections, the PL and IL receive input from the enthorinal and perirhinal cortices, whereas the dorsal prefrontal regions are targeted by afferents from the somatosensory and motor cortices. The mPFC also displays very strong intrinsic ipsilateral and bilateral connectivity (Vertes, 2004; Gabbott et al., 2005; Vertes et al., 2007; Hoover and

Vertes, 2007; reviewed in Euston et al., 2012; Courtin et al., 2013). The mPFC seems to display a dorso-ventral gradient, where ventral regions including the ventral PL and IL are specialised in autonomic/emotional control and dorsal regions including the AC and dorsal PL are specialised in the control for actions. The connectivity of dorsal and ventral mPFC is very similar with the exception that the dorsal mPFC has weaker connections with the autonomic/emotional centres and stronger connections with motor and pre-motor areas (Heidbreder and Groenewegen, 2003; Gabbott et al., 2005; Hoover and Vertes, 2007; Euston et al., 2012).

Cortical circuit organisation has been predominantly studied in sensory areas such as the visual and somatosensory cortices (Shepherd, 2009; van Aerde and Feldmeyer, 2013). A prominent feature of these areas is the presence of a clearly distinguishable granular layer 4. The granular appearance is due to the numerous small stellate or stellate-like neurons present in this layer (Steiger et al., 2004). The detailed analysis of the sensory cortex has resulted in a serial model of cortical circuit organisation that has served as a model for cortical circuits in general. In brief, layer 4 is the main target of thalamic input. This input is then forwarded to layer 2/3 where it is integrated with inputs arriving from other cortical areas. The activity is next relayed to layer 5 neurons, which are the main source of subcortical projections. Layer 5 neurons innervate those in layer 6, which then close the loop by projecting back to layer 4 and the thalamus. Finally, these neurons also display various patterns of inter- and intralaminar connectivity (Feldmeyer et al. 2002; Douglas and Martin 2004; Schubert et al. 2007; Thomson and Lamy, 2007; Feldmeyer 2012; Oberlaender et al. 2012; van Aerde and Feldmeyer, 2013). However, in contrast to the somatosensory cortex, the mPFC is agranular because it lacks a well-defined layer 4 (Gabbott et al., 1997). The organisation of neuronal circuits in agranular cortices is less understood (Shepherd, 2009; van Aerde and Feldmeyer, 2013). This is partially due to the lack of the canonical connection between layer 4 and 2/3 but also because the inputs into the mPFC are not as easy to control as sensory inputs. The analysis of the direction of information flow in agranular circuits is therefore less straightforward. However, studies on motor areas in the rodent frontal cortex suggest that synaptic circuits in agranular cortices might be organised around an excitatory pathway originating in layer 2/3 and targeting multiple classes of layer 5 projection neurons. Horizontal projections are present but appear less prominent than the layer 2/3 to layer 5 pathway (Weiler et al., 2008; Shepherd, 2009).

Despite the lack of a defined layer 4, the mPFC still displays strong reciprocal connectivity with the thalamus. In fact, historically, the prefrontal cortex has been defined on the basis of the inputs it receives from the mediodorsal (MD) nucleus of the thalamus (Rose and Woolsey, 1948; Fuster, 2008). More specifically, the PL and IL regions receive input from the medial segment of the MD, with the PL also receiving input from the lateral segment. The lateral segment also innervates the AC, whereas the PrCm receives input from the paralamellar segment. These MD projections terminate mainly in layers 2/3 of the mPFC (Uylings and van Eden, 1990; Courtin et al., 2013).

3.2.1 Cellular composition

The mPFC contains two main neuronal types – glutamatergic pyramidal neurons and GABA (γ-aminobutyric acid) releasing interneurons. The neurons are organised into five cortical layers: layer 1, 2, 3, 5 and 6. Pyramidal excitatory neurons constitute 84% of the total cell population of the mPFC and are located throughout all layers, except layer 1. They are spiny neurons, with long apical dendrites emerging from the top of the soma running perpendicularly to the pia surface until they bifurcate in layer 2 and terminate in layer 1 in a dendritic tuft (Gabbott et al., 1997; Vertes, 2004).

The population of GABAergic interneurons comprises the remaining 16% of mPFC neurons. These interneurons are located in all layers of the mPFC and are typically aspiny. On the neurochemical level interneurons are often categorised based on the calcium-binding proteins they express, such as parvalbumin (PV), calretinin (CAL) and calbindin (CB) as well as neuropeptides and their synthesising enzymes such as somatostatin (SOM), vasoactive intestinal peptide (VIP), cholecytokinin (CCK), nitric oxide synthase (NOS) and neuropeptide Y (NPY; Courtin et al., 2013; Markram et al., 2004). It is known that in the mPFC, CAL, PV and CB positive interneurons constitute 4.0%, 5.7% and 3.8% of all neurons respectively and together amount to approximately 80% of the GABA interneuron population (Gabbott et al., 1997).

3.2.2 The anatomy of the PL and its connectivity with the BLA and within the mPFC

The PL is located dorsally to MO in the anterior part of the mPFC, whereas in the more posterior sections it is located dorsally to the IL and ventrally to AC. The individual layers of the PL are more distinguishable than those of the IL but are less clearly defined than those of the AC. In mice, layer 1 is characterised by sparse cell density and is relatively thick,

measuring approximately 120 micrometres, whereas layer 2 is thin and densely packed with small cell bodies. Layer 1 contains several types of GABAergic interneurons that provide feed-forward inhibition onto pyramidal neurons. The transition from layer 2 to 3 is marked by a clear reduction in cell density. Together layer 2 and 3 measure approximately 160 micrometres. Layer 5 starts at 280 micrometres away from pia and stretches for approximately 285 micrometres. It contains medium to large pyramidal somata. Layer 6 can be separated into layer 6a and 6b, with layer 6b representing a thin layer of closely packed cells, many of which have horizontally elongated somata. The thickness of layer 6 is approximately 290 micrometres and it terminates at roughly 855 micrometres away from pia (taken from Poorthuis et al., 2013). After layer 6 there is a noticeable decline in cell body density after which the underlying white matter starts (Gabbott et al., 1997). The PL can be subdivided into dorsal and ventral PL, based on the characteristics of layer 2. In dorsal PL layer 2 is narrow and compact and it becomes broader and less compact in ventral PL. On the other hand, layers 3 and 5 are more compact in ventral PL (van de Werd et al., 2010).

PL connectivity with the BLA

BLA provides direct excitatory monosynaptic input into the PL. These connections are predominantly ipsilateral, displaying sparser projections to the contralataral hemisphere. BLA input to the PL terminates in two distinct horizontal bands one in layer 2 and one in layer 5/6. The dense terminal fields in layer 2 contain fine varicose axonal processes arising from vertically aligned axon collaterals projecting from layer 5. These collaterals also send off several varicose side branches as they travel through layer 3. BLA inputs are denser in ventral PL than in dorsal PL. The vast majority, namely 93-96% of the synapses are formed between axon terminals and dendritic spine heads, most likely being part of apical, basal and oblique dendrites of pyramidal neurons. The remaining synapses are made onto shafts of small to medium dendrites of both spiny and aspiny neurons as well as somata. Among the latter group is a subpopulation of PV-immunoreactive interneurons (McDonald, 1991; Bacon et al., 1996; Gabbott et al., 2006; Gabbott et al., 2012).

The PL also provides direct excitatory input back into the BLA. These projections are bilateral and target mainly the basal nuclei (BA) of the amygdala with some fibres innervating also the lateral nucleus (LA). The BLA-projecting neurons are located mainly in layers 2/3 and 5. The percent of neurons projecting to the BLA out of all projection neurons is

approximately 8%, 3%, 8% and 1% of layers 2, 3, 5 and 6 respectively (McDonald et al., 1996; Vertes, 2004; Gabbott et al., 2005).

PL connectivity within the mPFC

The PL is strongly interconnected with other regions of the mPFC: PrCm, AC, PL and IL. These connections are both ipsi- and contralateral and arrive from/target all layers of these areas. However, the exact density of these projections varies in the rostral/caudal axis. The majority of the synapses, 88-93%, are formed with dendritic spine heads. The remaining axon terminals form synapses with the shafts of fine dendritic processes. Presumably, some of them target interneurons. All axonal boutons form asymmetric (presumably excitatory) synapses with the postsynaptic target (Gabbott et al., 2003; Vertes, 2004; Hoover and Vertes, 2007).

3.3 mPFC and memory

The mPFC is known to be important for the consolidation, storage and retrieval of long-term memories. Several types of imaging studies were among the first to demonstrate the involvement of the mPFC in remote long-term memory. Remote (one month following behavioural training) but not recent (one day after training) memory retrieval was shown to result in increased expression levels of the immediate early genes *c-fos* and *zif268* (Frankland et al., 2004; Maviel et al., 2004), increased metabolic activity (Bontempi et al., 1999) and structural changes such as an increase in dendritic spine numbers (Restivo et al., 2009; Vetere et al., 2011). Furthermore, the lesioning or inactivation of the mPFC has also been shown to block remote memory recall across a wide range of behavioural tasks such as the radial arm maze (Maviel et al., 2004), Morris water maze (Teixeira et al., 2006), socially transmitted food preference (Lesburguères et al., 2011), conditioned taste aversion (Ding et al., 2008) trace eyeblink conditioning (Takehara et al., 2003; Oswald et al., 2010) and contextual fear conditioning (Frankland et al., 2004). Even though remote memory is often assayed at around 30 days after learning, the mPFC has been show to be involved in remote memory retrieval as late as 200 days following training (Quinn et al., 2008).

While the role of the mPFC in remote memory is well established, the involvement of the mPFC in recent memory has only become evident recently. Indeed, the mPFC has been shown to be necessary for recent memory retrieval in tasks that were learned only 1-2 days before testing. These include navigational tasks (Churchwell et al., 2010), object-place

association (Lee and Solivan, 2008) and a range of fear conditioning paradigms (Zhao et al., 2005; Blum et al., 2006; Corcoran and Quirk, 2007; Quinn et al., 2008).

The exact role played by the mPFC at recent and remote time points is, however, still unclear. Some accounts suggest that during recent memory retrieval the role of the mPFC is to represent context, events and responses while the hippocampus stores the exact mapping between them. In contrast, during remote memory recall, the mPFC might both represent and store the context-event-response mappings, while the hippocampus becomes disengaged (Euston et al., 2012). Other accounts suggest that the mPFC plays an active role in memory consolidation. Indeed, interfering with mPFC activity briefly after learning results in severe memory impairments when tested at later time points (Tronel and Sara, 2003; Tronel et al., 2004; Akirav and Maroun, 2006; Carballo-Márquez et al., 2007, 2009; Leon et al., 2010). For example, blocking plasticity mechanisms within the mPFC immediately following trace fear conditioning causes deficits in memory when tested 24 or 72 hours later (Runyan et al., 2004). Similar results have been obtained for inhibitory avoidance (Holloway and McIntyre, 2011; Zhang et al., 2011) and the consolidation of extinction of both fear and drug-related memories (Mamiya et al., 2009; Peters et al., 2009; Sotres-Bayon et al., 2009). There seems to be a specific time window during which the pharmacological disruption of mPFC activity can affect consolidation. This time window typically occurs 1-2 hours after learning and manipulations outside this window do not seem to impair long-term memory formation (Tronel and Sara, 2003; Carballo-Márquez et al., 2007).

3.4 mPFC and fear conditioning

Through its anatomical connections with the BLA, the hippocampus and other subcortical areas important for emotional processing, the mPFC is in a strategic position to exert executive control over emotional responses and to create long lasting associations between threatening cues and environmental contexts (Euston et al., 2012). Early indications of the importance of the mPFC in fear memory come from the 1950s when it was shown that post-conditioning frontal lobotomy abolished fear responses in both monkeys and rats (Streb and Smith, 1955; Waterhouse, 1957). Since then a vast number of lesion and inactivation studies have further confirmed the involvement of the mPFC in fear memory acquisition, consolidation, expression and extinction, although the exact contribution of individual mPFC subregions remained controversial for a while (Morgan et al., 1993; Morgan and LeDoux, 1995; Morrow et al., 1999; Vouimba et al., 2000; Morgan et al., 2003; Bissière et al., 2008).

Results from functional histochemical studies provided further support for the involvement of the mPFC in fear learning. Elevated expression levels of the immediate early genes *c-fos* and *zif268* were shown to occur following contextual (Beck and Fibiger, 1995; Thomas et al., 2002; Frankland et al., 2004; Tulogdi et al., 2012) and cued fear conditioning as well as fear extinction learning (Morrow et al., 1999; Herry and Mons, 2004; Santini et al., 2004).

In recent years, the specific roles played by each of the individual mPFC subregions in fear conditioning and extinction became more evident. The AC has been shown to be important for fear memory acquisition and storage (Frankland et al., 2004; Tang et al., 2005; Bissière et al., 2008). Indeed, the genetic and pharmacological inhibition of the GluN2B glutamate receptors in the AC impairs the acquisition of contextual fear memory (Zhao et al., 2005; Einarsson and Nader, 2012), while the infusion of the protein synthesis inhibitor anisomycin impairs consolidation and reconsolidation of both recent and remote contextual fear memories (Einarsson and Nader, 2012). The inactivation of the PL consistently results in the impairment of fear memory expression without any effect on memory extinction (Vidal-Gonzales et al., 2006; Corcoran and Quirk, 2007; Laurent and Westbrook, 2009; Sierra-Mercado et al., 2011; Fenton et al., 2014). Its involvement in fear acquisition and consolidation has also been suggested (Lauzon et al., 2009; Choi et al., 2010). Fear renewal after extinction also depends on PL activity (Knapska and Maren, 2009). In contrast to the PL, the inactivation of the IL seems to selectively impair fear extinction learning while having no effect on fear expression (Laurent and Westbrook, 2009; Sierra-Mercado et al., 2011). Indeed, successful consolidation of extinction memory has been shown to result in increased expression levels of *c-fos* specifically in the IL (Knapska and Maren, 2009). In contrast, when extinction memory is impaired the expression of *c-fos* and/or *zif268* in the IL is reduced (Hefner et al., 2008; Muigg et al., 2008). Moreover, post-extinction memory retrieval is impaired following the infusions of NMDA (N-methyl-D-aspartate; Burgos-Robles et al., 2007; Sotres-Bayon et al., 2009), dopamine (Pfiffer and Fendt, 2006; Hikind and Maroun, 2008) and noradrenergic receptor antagonists (Mueller et al., 2008; Mueller and Cahill, 2010) into the IL, or when protein synthesis in this brain region is blocked (Santini et al., 2004). Finally, the stimulation of IL neurons inhibits freezing to the conditioned tone (Milad et al., 2004). In summary, it is now believed that the AC plays a role in the formation of fear memories, the PL mediates the expression of conditioned fear, whereas the IL is involved in extinction learning and memory (Courtin et al., 2013).

3.4.1 mPFC and its partners in the fear circuit

The mPFC does not play a unitary role in mediating fear learning and memory but does so through the communication with its partners in the fear circuit (Kim and Jung, 2006; Marek et al., 2013). Below I review how the mPFC interacts with the hippocampus and the BLA and how this might be important for acquiring, storing and retrieving fear memories.

mPFC and the hippocampus

It is known that the interaction between the mPFC and the hippocampus is important for memory formation in a wide range of spatial behavioural paradigms (reviewed in Euston et al., 2012). One way in which information between these two structures might be transferred and eventually stored is via neural oscillations (Buzsaki and Draguhn, 2004; Buzsaki et al., 2012). Indeed, the mPFC shows strong theta coherence with the hippocampus. Approximately 50% of the cells in the mPFC are phase locked to hippocampal theta rhythm (3-12 Hz) during spatial memory tasks (Hyman et al., 2005; Jones and Wilson, 2005; Siapas et al., 2005; Sirota et al., 2008) and approximately 30% of putative principal neurons and 45% of interneurons in the mPFC display firing modulation as a function of hippocamal theta activity (Sirota et al., 2008). Moreover, this hippocampo-mPFC theta coherence causes a significant reorganisation of the dynamics of neural ensembles in the mPFC resulting in a synchronised activation of groups of cells (Benchenane et al., 2010). Most likely, during behaviourally relevant information, hippocampal theta rhythms modulate mPFC interneurons, which in turn control principal cell dynamics leading to their synchronisation and the formation of cell assemblies (Benchenane et al., 2011). This is in agreement with the recent study by Courtin et al., (2014) showing that the inhibition of PV interneurons in the dorsal mPFC mediates the resetting of the local theta oscillations phase thus resulting in enhanced synchronisation of pyramidal neurons and increased output efficiency (Courtin et al., 2014).

This hippocampo-mPFC theta coherence has been shown to be necessary for selecting information that is relevant for long-term storage. Indeed, there is evidence that theta oscillations synchronise mPFC neurons in a learning-dependent manner and the neuronal activity of the mPFC modulates sensory information transfer during learning (Paz et al., 2008, 2009). Additionally, the theta-coherence of the hippocampo-prefrontal network is higher during anxiety-related behaviours (Adhikari et al., 2010). More importantly during the retrieval of conditioned fear, the theta coupling increases with high specificity between the BLA-CA1-mPFC, whereas it decreases during extinction learning (Lesting et al., 2011). Also,

it is known that neurons in the mPFC display oscillations in the theta band when recorded during freezing (Narayanan et al., 2007; Lesting et al., 2011). Finally, the activated mPFC cell assemblies displaying high coherence with the hippocampus seem to be preferentially replayed during hippocampal sharp-wave ripple oscillations, which has been linked to processes like memory consolidation (Euston et al., 2007; Peyrache et al., 2009; Benchenane et al., 2010; Popa et al., 2010; Logothetis et al., 2012).

mPFC and the BLA

The mPFC and the BLA are reciprocally connected and the interaction of the two structures is necessary for the acquisition, expression and extinction of conditioned fear memory (McDonald, 1991; Bacon et al., 1996; McDonald et al., 1996; Quirk et al., 2003; Vertes, 2004; Gabbott et al., 2005, 2006; Stevenson, 2011; Vouimba and Maroun, 2011). Indeed, fear conditioning and fear reinstatement result in an increase in the evoked field potential amplitudes at the mPFC-BLA pathway. This increase correlates positively with freezing levels during memory retrieval. Fear extinction causes a synaptic depression at this pathway, whereas fear reinstatement following extinction induces its re-potentiation (Vouimba and Maroun, 2011). Moreover, when the communication between the BLA and the dorsal mPFC is disrupted, the expression of conditioned fear memory is impaired (Stevenson, 2011).

The BLA sends glutamatergic projections to the central nucleus (CEA) of the amygdala, which is the main source of amygdala output through its connections with the brain stem and the hypothalamus (Sah et al., 2003; Kim and Jung, 2006; Pape and Paré, 2010). The stimulation of the mPFC decreases the responsiveness of CEA output neurons to synaptic activation from the BLA, showing that the mPFC could mediate fear responses by directly controlling amygdala output (Quirk et al., 2003). This decrease in CEA responsiveness is most likely mediated by IL neurons (Paré et al., 2004; Vidal-Gonzales et al., 2006; Courtin et al., 2013).

Further evidence for the interaction of the mPFC and the BLA during high and low fear states comes from functional histochemical studies. Neurons in the PL that project to the BLA have increased levels of *c-fos* expression following the renewal of conditioned fear. In contrast, *c-fos* expression in the BLA-projecting IL neurons is increased following fear extinction (Orsini et al., 2011). While PL neurons might promote fear expression and renewal through their projections to the principal neurons in the BLA, the IL most likely inhibits fear behaviour through its projections to the local inhibitory neurons in the LA as well as the

intercalated cell masses (ITC) and CEA regions of the amygdala (Paré et al., 2004; Vidal-Gonzales et al., 2006; Courtin et al., 2013). Furthermore, based on immediate early gene expression two distinct neuronal populations were identified in the LA: one, in which increased *c-fos* expression levels result from the renewal of conditioned fear and another one in which *c-fos* expression correlates with low freezing following the retrieval of extinction memory. The former neuronal population is innervated by the ventral hippocampus and the PL, whereas the latter receives inputs mainly from the IL (Knapska et al., 2012). Finally, neurons in the BA (basal nuclei of the amygdala) that project to the PL and the ventral hippocampus show elevated *c-fos* expression following states of high fear. Extinction on the other hand causes an increase in *c-fos* expression uniquely in the IL-projecting BA neurons (Senn et al., 2014). More importantly, however, the inhibition of the BA-PL pathway improves fear extinction learning while the optogenetic inhibition of the BA-IL pathway impairs extinction (Senn et al., 2014).

3.4.2 PL and fear conditioning

The PL is thought to play a role in the learning and consolidation of conditioned fear but its involvement seems most important for mediating fear expression (Lauzon et al., 2009; Choi et al., 2010; Sotres-Bayon and Quirk, 2010; Sierra-Mercado et al., 2011; Courtin et al., 2013). The PL receives direct input both from the hippocampus (McDonald, 1991; Gabbott et al., 2002, 2006; Hoover and Vertes, 2007) and the BLA and is therefore in a perfect position to mediate fear expression through the integration of these two input sources. Indeed, certain neurons in the PL have been shown to be responsive to both ventral hippocampal and BLA inputs (Sotres-Bayon et al., 2012).

The microstimulation of the PL increases freezing to the conditioned tone and prevents extinction learning, whereas the stimulation of adjacent mPFC regions, AC or PrCm, has no effect on freezing levels (Vidal-Gonzalez et al., 2006). Furthermore, the pharmacological inactivation of the PL with the Na^+ channel blocker tetrodotoxin (TTX) just before fear retrieval takes place significantly reduces freezing to both the conditioned contexts and tones. While this same inactivation prior to conditioning does not seem to prevent fear learning it does, however, reduce freezing levels during the fear conditioning session (Corcoran and Quirk, 2007). Furthermore, neuronal activity in the PL correlates highly with fear expression, but not extinction. Approximately 20% of neurons in the PL are tone responsive and some of them show a sustained increase in the excitatory response to the conditioned tone. This

increase is correlated with high-fear states (conditioning and early extinction) but not with low-fear states (habituation and late extinction). In fact, the increase in tone-responsiveness is reduced following fear extinction. Moreover, the exact timing of PL activity matches freezing responses on a second by second scale. An increase in PL activity starts as early as 100 milliseconds after the tone onset and it precedes freezing behaviour by approximately 2 seconds. This strongly suggests that the activity of the PL might directly mediate freezing behaviour – perhaps by sustaining the fear responses initiated by the amygdala (Burgos-Robles et al., 2009).

4. Intrinsic Plasticity in Learning and Memory

In Chapter 4 I review what is known about learning-induced changes in intrinsic excitability with a special focus on changes induced by fear conditioning. I start this chapter by defining intrinsic plasticity and introducing the major classes of voltage-gated ion channels and Ca^{2+} activated K^+ channels, which are important for mediating changes in neuronal excitability. We investigated changes in intrinsic excitably as measured at the somatic level. The plasticity of dendritic excitability was not within the scope of this project. However, active dendritic properties are important contributors in shaping the neuron's overall excitability levels. For more information on the plasticity of dendritic excitability please refer to our book chapter: Szlapczynska et al., 2014, *Plasticity and Pathology of Dendritic Intrinsic Excitability* (see Appendix). Certain information discussed in the book chapter is also included in Section 4.2 Ion channels involved in regulating intrinsic excitability and Section 4.3 Plasticity of intrinsic excitability.

4.1 Overview

Learning and memory are most frequently studied as a change in the efficacy of synaptic transmission. However, it is now clear that learning can also induce non-synaptic forms of plasticity such as changes in the intrinsic excitability of a neuron. Intrinsic plasticity can be defined as a change in the neuron's electrical properties induced by various neuromodulators, activity patterns, disease states, or learning paradigms (Zhang and Linden, 2003; Frick and Johnston, 2005; Disterhoft and Oh, 2006; Mozzachiodi and Byrne, 2009). This change is mediated by a wide range of voltage-gated Na^+, Ca^{2+}, K^+ and hyperpolarisation activated and cyclic nucleotide-gated (HCN) channels, as well as voltage-independent ion channels present in the neuronal membrane (Migliore and Shepherd, 2002; Magee, 2008; Nusser, 2012). The exact kinetics and expression levels of these channels are characteristic for a given neuronal type and its sub-cellular compartments (e.g. soma versus dendrites). Any changes in the biophysical properties and/or distribution of these channels will alter the excitability of the neuronal membrane (Hille, 2001; Frick and Johnston, 2005). Intrinsic plasticity can take place anywhere along the cellular membrane causing a neuron-wide change in excitability, or it can occur locally and remain restricted to individual dendritic branches. Changes induced in the somatodendritic compartment typically affect the integration and propagation of synaptic inputs whereas those occurring in the axon modulate action potential properties (AP) and synaptic transmission.

The study of intrinsic plasticity is particularly useful in the context of learning and memory because it can affect a wide range of neuronal functions including the presynaptic release of neurotransmitters, the way synaptic inputs are integrated, the AP output at the soma and the active back-flow of information into the dendrites. Additionally, it has been suggested that it serves as a mechanism for metaplasticity, homeostatic regulation of neuronal activity, memory allocation or it even acts as part of the memory trace itself (Helmchen et al., 1999; Zhang and Linden, 2003; Frick et al., 2004; Frick and Johnston, 2005; Chen et al., 2006; Disterhoft and Oh, 2006; Zhou et al., 2009; Mozzachiodi and Byrne, 2010 Benito and Barco, 2010; Xu et al., 2012; Szlapczynska et al., 2014*)*.

4.2 Ion channels involved in regulating intrinsic excitability

Neurons contain a vast number of ion channels, which play an important role in shaping changes in the excitability of the neuron. Ion channels are pore forming membrane proteins and are the most fundamental excitable elements in the neuronal membrane. These channels are specifically responsive to incoming stimulation such as membrane potential change, neurotransmission and neuromodulation, chemical signalling or mechanical deformation, to name just a few. When ion flux occurs across the membrane, it has an immediate effect on the membrane potential leading to the activation of voltage-sensitive ion channels. This process of ion channel activation is, therefore, regenerative and self-propagating. Ion channels are present in membranes of all cells and play a critical role in regulating many aspects of their function. For example they are responsible for shaping the cell's resting membrane potential (RMP), controlling the flow of ions across the neuronal membrane including the messenger Ca^{2+} ions, regulating the electrical signals generated by the cell as well as controlling the cell's volume (Hille, 2001). The quest for investigating the distribution and properties of voltage-gated ion channels can be dated back to the 1950s. That is when Alan Hodgkin and Andrew Huxley first described a model that could accurately predict the shape of the AP generated and propagated by the squid giant axon (Hodgkin and Huxley, 1952). Since then electrophysiological and molecular studies unveiled the complex distribution patterns and properties of various families of ion channels in the neuronal membrane (Lai and Jan, 2006; Migliore and Shepherd, 2002; Catterall et al., 2012; Szlapczynska et al., 2014). Below I provide an overview of the voltage-gated ion channels that are present in mammalian pyramidal neurons of the neocortex. A description of Ca^{2+} activated K^{+} ion channels is also included.

Voltage-gated ion channels fall into a number of broad categories based on their selectivity for certain ions, for example K^+, Na^+, Ca^{2+} (Kv, Nav, Cav, respectively) and non-selective cation channels activated by hyperpolarisation (i.e. hyperpolarisation activated and cyclic nucleotide-gated channels, HCN; Figure 4.1). All mammalian voltage-gated ion channels contain one or four transmembrane pore-forming proteins. More specifically, Nav and Cav channels are formed by a single α-subunit (Nav) or α_1-subunit (Cav) containing four transmembrane repeats (domains I-IV), whereas Kv and HCN are formed by four α-subunits, each with a single domain. In the case of Kv and HCN channels, the four α-subunits can co-assemble to form both homo- and heterotetrameric pore-forming subunits, increasing their molecular diversity (Lujan, 2010; Lewis and Chetkovich, 2011) Each domain comprises six transmembrane segments (S1-S6). The S4 segment in each domain detects changes in membrane potential whereas segments S5 and S6 and the re-entrant pore loop between them form the lining of the pore. These pore-forming subunits are associated with auxiliary subunits (α2, β, γ or δ), as well as a range of other molecules, such as scaffolding proteins, which serve to modulate the channels' properties or their subcellular location. Differential splicing, the formation of heteromers and additional post-translation modifications further extend the diversity of voltage-gated ion channels (Migliore and Shepherd, 2002; Vacher et al., 2008; Lujan, 2010).

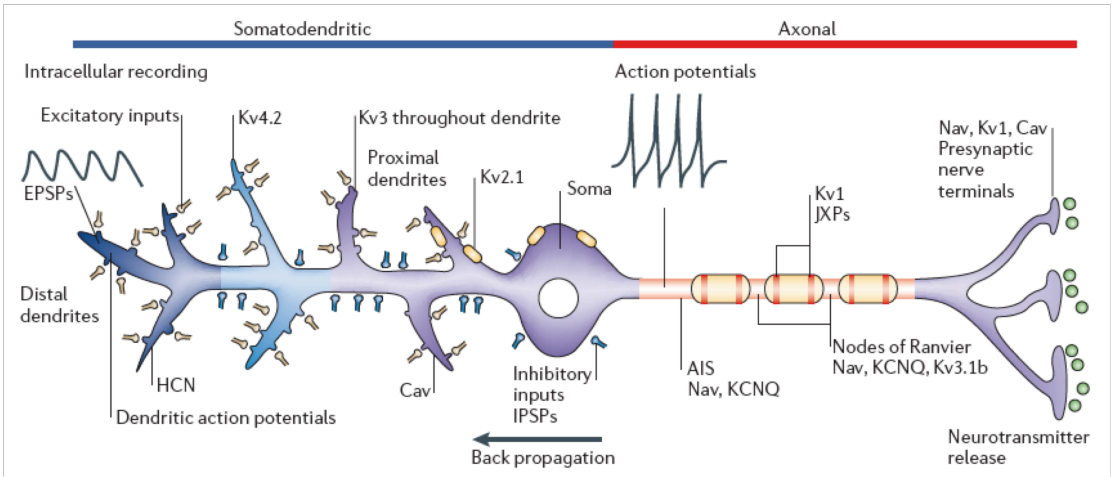


Figure 4.1 General localisation of voltage-gated ion channels in a model neuron. Taken from Lai and Jan, 2006.

4.2.1 Nav channels

Nav channels allow for a fast depolarising inward current to enter the cell and thus are the primary channels responsible for membrane depolarisation and the generation and propagation of AP in the axon (Hodgkin and Huxley, 1952) as well as the back-propagation of APs back into the dendrites (Stuart and Sakmann, 1994; Johnston et al., 1996; Stuart et al.,1997a, 1997b). They also allow for the generation of Na^+ mediated dendritic spikes and the non-linear integration of synaptic potentials (Stuart et al., 1997b; Larkum et al., 2007; Larkum et al., 2009). In mammals, ten genes are known to encode the α-subunits of Nav channels - Nav1.1 to Nav1.9. Of the ten known Nav family members, four subunits (Nav1.1, 1.2, 1.3 and 1.6) are expressed in the rodent/human brain (Goldin et al., 2001, Trimmer and Rhodes, 2004).

The Nav1.2 subunits are expressed specifically in the axon and its terminals and are thought to be the major components of the AP conductance mechanisms as well as important regulators of neurotransmitter release in the terminals (Westenbroek et al., 1989). Nav1.1 and Nav1.3 show a somatodendritic distribution, with Nav1.3 having the highest expression levels in the embryonic and early postnatal brain. Finally, Nav1.6 is present both in the somatodendritic compartment and in the axon (Goldin et al., 2001; Trimmer and Rhodes, 2004). Nav channels are sensitive to a large number of neurotransmitters, which have been shown to alter their function. The phosphorylation of Nav channels has important functional consequences as it can result in a reduction of Na^+ currents. This mainly occurs via the phosphorylation of the α-subunits by the cAMP (cyclic adenosine monophosphate) dependent protein kinase A (PKA) and protein kinase C (PKC) or dephosphorylation by the Ca^{2+} regulated phosphatase calcineurin and protein phosphatase (Frick and Johnston, 2005; Cantrell and Catterall, 2001).

Although the majority of the current generated by Nav channels is fast, Nav channels can also generate a persistent noninactivating or slowly inactivating current I_{NaP} (Crill 1996; Kiss 2008). This current constitutes only a small fraction of the transient Na^+ current but it has significant physiological consequences. It is implicated in setting the membrane potential, regulating repetitive firing and enhancing synaptic transmission. It is not clear which subunits generate this type of current (Kiss, 2008).

4.2.2 Cav channels

Cav channels are important regulators of neuronal excitability. They are expressed in the somata, dendrites and axon terminals. They mediate Ca^{2+} influx into the cell following membrane depolarisation and play an active role in the amplification of incoming synaptic signals, generation of dendritic Ca^{2+} spikes, the activation of Ca^{2+} gated K^+ channels, signalling resulting from back-propagating APs and synaptic plasticity. Cav channels also mediate a range of intracellular processes such as Ca^{2+} dependent enzyme activation, gene transcription and neurotransmitter secretion (Vacher et al., 2008; Catterall, 2011). For those reasons, Cav channels create an important link between neuronal excitability, physiological events within the cell and synaptic plasticity. Cav channels can be classified into three families: Cav1-3 giving rise to five different current types: L-type (Cav1), P/G-type (Cav2.1), N-type (Cav2.2), R-type (Cav2.3) and T-type (Cav3; Lai and Jan, 2006).

Cav1 or L-type channels are expressed in the somata and dendrites where they play an important role in somatodendritic signalling. They require a high voltage for activation, conduct large currents and display slow inactivation kinetics. On the other hand, Cav3 or T-type channels are activated at much more negative membrane potentials, have small single channel conductance, inactivate rapidly and deactivate slowly. They play a role in regulating Ca^{2+} permeability around RMP and AP firing. Finally, Cav2 require a high voltage for activation and display fast deactivation kinetics and moderate to slow inactivation kinetics. Apart from playing a role in somatodendritic Ca^{2+} influx they also regulate fast neurotransmitter release from presynaptic terminals (Trimmer and Rhodes, 2004; Vacher et al., 2008; Catterall, 2011). Cav channels are modulated by phosphorylation, G-proteins and by Ca^{2+} itself (Catterall, 2000).

4.2.3 K^+ channels

K^+ channels are the most important regulators of intrinsic excitability and are the largest and most diverse ion channel group. In general K^+ play an important role in dampening neuronal excitability by providing outward currents. Their exact functional role, however, depends on their specific biophysical properties such as activation/inactivation kinetics, voltage-dependence, activation by intracellular ions and second messengers (Yuan and Chen, 2006). There are over 100 genes in the mammalian genome encoding the K^+ channel α- and auxiliary subunits (Gutman et al., 2005; Yuan and Chen, 2006). K^+ channels can be subdivided into several groups based on their pore forming α-subunits – voltage-gated (Kv),

Ca^{2+} activated, inward rectifying (Kir3/GIRK) and two-pore (K_{2P}) channels. In contrast to Kv and Ca^{2+} activated K^+ channels, K_{2P} and Kir3 are formed by four and two transmembrane domains respectively (Lujan, 2010) and will not be discussed any further.

Kv channels

Kv channels represent the largest and most diverse group of voltage-gated ion channels (Vacher et al., 2008, Lujan, 2010). The mammalian Kv α-subunits are encoded by approximately 40 genes, which can be grouped into 12 families *Kv1-Kv12* (Gutman et al., 2005). The prototypic Kv subunits were initially identified by mutant screens in Drosophila rather than by biochemical analysis and they were categorised into 4 families (Kv1-Kv4) based on their Drosophila homologues *Shaker, Shab, Shaw, Shal*. More recently, α-subunits forming Kv5-Kv12 subfamilies have been discovered. Consequently, the genes encoding the α-subunits are known under these names: *Shaker*/Kv1/KCNA; *Shab*/ Kv2/KCNB; *Shaw*/Kv3/KCNC; *Shal*/Kv4/KCND; Kv5/KCNF; Kv6/KCNG; Kv7/KNCQ; Kv8/KCNV; Kv9/KCNS; Kv10/KCNH; Kv11/KCNH; and Kv12/ KCNH.

The expression pattern of Kv.1 is limited mainly to the axon and nerve terminals, Kv2 and Kv4 show a somatodendritic distribution, whereas Kv3 is expressed both in the axonal and somatodendritic compartments. Kv7 expression is mainly axonal, with some accounts of also somatodendritic localisation. Information about the subcellular distribution of Kv5-6 and Kv8-12 is limited (reviewed in Vacher et al., 2008). Depending on their activation kinetics Kv channels can be categorised based on the types of currents they generate: transient (Kv1.4, Kv3.3-Kv3.4, Kv4), sustained (Kv1.1-Kv1.3, Kv1.5-Kv1.6) or delayed rectifying (Kv2, Kv7; reviewed in Lujan, 2010).

One of the most studied K^+ channels is the rapidly activating and inactivating dendritic A-type K+ channel. Most of our knowledge about this channel and the current it generates comes from studies on the hippocampus. In the neocortex, the A-type K^+ current is generated predominantly by Kv4.2 and Kv4.3 subunits with some contribution from Kv1.4 (Norris and Nerbonne, 2010). These currents regulate AP repolarisation, repetitive firing, synaptic integration and AP backpropagation (Frick et al., 2003; Johnston et al., 2003; Cai et al., 2004; Yuan and Chen, 2006). Kv4.2 is regulated by a number of intracellular signalling pathways and kinases, including PKA, PKC, MAPK (Mitogen – activated protein kinase) and CaMKII (Ca^{2+}/calmodulin – dependant protein kinase; Schrader et al., 2002; Rosenkranz et al., 2009). The activation of PKA, PKC and MAPK by various neuromodulators results in a decrease of

the A-type K^+ channel activity (Hoffman and Johnston, 1998, 1999; Yuan et al., 2002), whereas CaMKII activation alters the channel's surface expression (Varga, 2004).

Kv7 channels are slow delayed rectifiers that are activated at subthreshold levels and play a role in maintaining the RMP and reducing excitability. Kv7 channels are most known for underlying the M-type current, a slowly activating and inactivating current that is modulated by G-proteins. The main subunits underlying the M-type current are Kv7.2 and Kv7.3. M-type K^+ plays a critical role in controlling the subthreshold membrane excitability, the generation of theta-frequency oscillations, the regulation of interspike intervals and the neuron's responsiveness to synaptic inputs (Wang et al., 1998; Guan et al., 2011). Moreover, Kv7 channels regulate the dynamics of neuronal firing (Rogawski, 2000; Peters et al., 2005) and have been shown to contribute to the generation of fast AHP (fAHP) occurring after a single AP (Brown and Passmore, 2009; Santini et al., 2010). Kv7 channels are also readily phosphorylated. PKA activation for example, enhances M-type currents, whereas the activation of the tyrosine kinase results in a slow inhibition of Kv7.2/Kv7.3 expression (reviewed in Park et al., 2008)

Kv1 channels that are expressed mainly in the axon and nerve terminals are likely to be the channels generating the dendrotoxin sensitive D-type K^+ current. D-type K^+ current is a fast-activating, slow-inactivating current that plays a role in controlling the AP threshold, waveform, firing frequency, presynaptic neurotransmitter release and synaptic efficacy (Bekkers and Denaley, 2001; Guan et al., 2007a; Kole et al., 2007; Shu et al., 2007).

Channels Kv1, Kv2 and Kv7 all contribute to somatic currents in neocortical pyramidal neurons. Out of the three, the Kv2 component accounts for the largest, about 60% of the total Kv current during large voltage steps (Guan et al., 2013). The Kv2 channels activate slowly at depolarised membrane potentials (Guan et al., 2007b) and due to their slow kinetics their major role is to regulate repetitive firing, especially at higher frequencies (Malin and Nerbonne, 2002; Johnston et al., 2008, Guan et al., 2013).

Kv3 family members display rapid activation kinetics at voltages more positive than -10 mV and very rapid inactivation kinetics. Their functional role is to facilitate sustained high frequency firing. They are therefore highly expressed in fast spiking neurons, such as neocortical or hippocampal interneurons (Vacher el al, 2008).

Ca^{2+} activated K^+ channels

Ca^{2+} gated K^+ channels are activated by an increase in the intracellular concentrations of Ca^{2+}. Their opening causes membrane hyperpolarisation and a decrease in neuronal excitability. The main Ca^{2+} activated K^+ channels expressed in the brain are the small-conductance voltage-insensitive K^+ channels (SK), with a single channel conductance of 2-20pS, and the big-conductance K^+ channels (BK), which are activated by both intracellular Ca^{2+} and voltage and have a single channel conductance of 200-400pS (Sah and Faber, 2002). SK channels are formed by three subunits SK1-SK3 and they are sensitive to apamin (found in bee venom) and bicuculline, which distinguishes them from BK channels (Sah and Faber, 2002; Wei et al., 2005, Yuan and Chen, 2006; Lujan, 2010). BK channels play a role in rapid repolarisation of APs and the fAHP, which lasts 2-5 milliseconds and occurs immediately after a single AP. Also, M-type and A-type currents are thought to contribute to fAHP. The fAHP plays a role in AP repolarisation and broadening, and controls spike frequency adaptation (Faber and Sah, 2003; Disterhoft and Oh, 2006; Yuan and Chen, 2006). SK channels on the other hand are thought to underlie the medium AHP (mAHP) currents that occur after a single AP or a burst of APs. The mAHP has a much slower decay time than the fAHP and lasts from 50-200 milliseconds (Stocker et al., 2004). In addition to fAHP and mAHP, the AHP can also have a third slow component (sAHP) that typically occurs after a burst of APs and decays over the course of seconds. The exact Ca^{2+} activated K^+ channel underlying this conductance has not yet been identified (Disterhoft and Oh, 2006, Andrade et al., 2012).

In the cortex, SK channels respond to the Ca^{2+} induced Ca^{2+} release triggered by the activation of muscarinic receptors (Yamada et al., 2004; Gulledge et al., 2007) or metabotropic glutamate receptors (Hagenston et al., 2008). In the prefrontal cortex the block of SK channels improves working memory (Brennan et al., 2008). In L5 pyramidal neurons of the mPFC SK channels regulate excitatory synaptic transmission through an interaction with NMDA (N-methyl-D-aspartate) receptors and Cav channels (Faber, 2010). Both SK and BK channels are present in the soma and dendrites of pyramidal cortical neurons (Sailer et al., 2004; Benhassine and Berger, 2005; Sausbier et al., 2006). However, SK1 and SK2 are highly expressed in the neocortex with SK3 showing higher expression levels in the subcortical regions (Stocker et al., 2004).

4.2.4 HCN channels

HCN channels differ a little bit from other voltage-gated ion channels as they are activated by hyperpolarisation and deactivated by depolarisation. They belong to the superfamily of K^+

channels, however, they are non-selective cation channels as they allow for a mixed K^+/Na^+ current to enter the cell. This hyperpolarisation-activated current is called the I_h current. HCN are directly modulated by cAMP. Because HCN channels can generate rhythmic activity in neurons and cardiac cells, they are often referred to as 'pacemaker channels'. I_h plays a wide range of important functions in neurons: they contribute to the RMP, act as resonance conductors, suppress temporal and spatial summation of synaptic inputs and decrease the efficacy of backpropagating AP. Most of HCN activity results in a reduction of dendritic excitability. However, I_h also causes rebound depolarisations following membrane hyperpolarisations that contribute to the generation of dendritic plateau potentials or spikes (Lujan, 2010; Lewis and Chetkovich, 2011).

HCN channels are composed of four subunits HCN1-HCN4. The functional properties of these channels are determined by the subunits that form them. For example, HCN1 subunits respond much faster to hyperpolarising potentials and display faster activation kinetics than HCN2-4. Out of all the subunits, HCN4 require the strongest hyperpolarisation and are the slowest to activate. On the other hand, HCN4 subunits are very sensitive to the presence of cAMP, whereas the responsiveness of HCN1 to cAMP is minimal. cAMP activity shifts the voltage activation of HCN channels to more depolarised potentials, which enhances their activation at RMP. In the neocortex, the two major subunits responsible for generating the I_h current are HCN1 and HCN2 and they show a non-uniform gradient of distribution increasing in density with distance from soma. HCN3 and HCN4 subunits are more concentrated in subcortical regions. HCN channels are also, although to a lesser degree, expressed in the axon (Pape, 1996; Migliore and Shepherd, 2002; Notomi and Shigemoto, 2004; Robinson & Siegelbaum, 2003; Kole et al., 2006).

4.3 Plasticity of intrinsic excitability

4.3.1 EPSP-spike potentiation

Synaptic plasticity involves the strengthening or weakening of the synaptic connection between two neurons, whereas intrinsic plasticity is the change of electrical properties within a single neuron. Despite being distinct, the two processes however act together to shape information transfer along the dendritic tree as well as neuronal output. For example, in the hippocampus the induction of long-term potentiation (LTP) can result in an enhanced probability that the postsynaptic neuron will fire APs in response to subsequent excitatory postsynaptic potentials (EPSPs). This phenomenon is known as the EPSP-to-spike

potentiation (E-S potentiation; Bliss and Lomo, 1973). It is now believed that E-S potentiation occurs not only due to an altered balance between excitation and inhibition following LTP induction (Abraham et al., 1987; Chavez-Noriega et al., 1989) but also requires intrinsic changes in both the post and presynaptic neurons (Taube and Schwartzkroin, 1988; Daoudal et al., 2002). For example, LTP induction in the CA1 increases EPSP summation through a downregulation of HCN channels (Wang et al., 2003), which are known to reduce the temporal summation of synaptic inputs (Magee, 2000; Poolos et al., 2002). Any change in the summation of synaptic inputs can in turn alter the spiking activity of the neuron (Magee, 1999, 2000). Indeed, LTP induction results in a subsequent increase in intrinsic excitability mediated by a decrease in AP threshold (Xu et al., 2005; Frick et al., 2004; Chen et al., 2006). LTD on the other hand, has been shown to reduce both pre and postsynaptic neuronal excitability (Daoudal et al., 2002; Li et al., 2004).

4.3.2 AP backpropagation

Action potentials are typically initiated in the axon initial segment and travel forward along the axon. However, in certain neuronal types such as the hippocampal CA1 and cortical pyramidal neurons they can also actively backpropagate into the dendrites thanks to the presence of Na^+ channels in the neuronal membrane (Stuart and Sakmann, 1994; Johnston et al., 1996; Stuart et al., 1997a, 1997b). The exact degree and manner of this propagation depends on the dendritic morphology (Vetter et al., 2001; Schaefer et al., 2003), the presence and activity of other voltage-gated ion channels distributed along the dendritic tree, prior neuronal activity and/or neuromodulation (Spruston et al., 1995; Stuart et al., 1997b; Stuart and Häusser, 2001; Frick et al., 2004; Hoffman and Johnston, 1999; Gentet and Williams, 2007). For example, LTP induction can also be accompanied by a persistent and local NMDA receptor dependent increase in dendritic excitability manifested by an augmentation of the backpropagating AP amplitude and an accompanying boost in Ca^{2+} signals (Frick et al., 2004). The resulting increase in dendritic excitability, which is mediated by A-type K^+ channels, favours the backpropagation of APs into the potentiated dendritic region (Watanabe et al., 2002; Frick et al., 2004).

Once triggered, backpropagating AP provide feedback information into the dendrites about axonal output, they can be implicated in the generation of dendritic spikes, facilitate synaptic integration and play a role in Hebbian synaptic plasticity (Waters et al., 2003; Sjöström et al., 2008). For example, when appropriately timed with EPSPs, backpropogating

APs can induce long-term potentiation (LTP) and depression (LTD) through spike-timing dependent plasticity protocols (STDP; Magee and Johnston, 1997, Letzkus et al., 2006; Sjöström and Häusser, 2006; for review on STDP see Dan and Poo, 2006).

4.3.3 Synaptic integration

Cortical pyramidal neurons possess elaborate dendritic arbours that receive and integrate excitatory and inhibitory inputs of varying amplitudes from numerous other neurons to give rise to cell-type specific firing patterns. The plasticity of dendritic integration largely depends on both the passive and active dendritic properties. Voltage-gated Na^+, A-type K^+, Ca^{2+} and HCN channels can all influence or be influenced by EPSPs (Stuart et al., 1997b; Magee, 2000; Williams and Stuart, 2003a; Gulledge et al., 2005; Spruston, 2008). HCN channels are of particular importance for subthreshold integration due to their specific activation/deactivation kinetics as well as their non-uniform somatodendritic gradient of distribution (Williams and Stuart, 2000a; Berger et al., 2001; Kole et al., 2006). Because their density increases with distance from soma in CA1 and neocrtical L5 neurons, they have the greatest impact on the summation of distal synaptic inputs (Magee 1998; 1999, 2000; Williams and Stuart, 2000a; Lörincz et al., 2002). Also, due to their specific kinetics, their activation reduces the duration of inhibitory postsynaptic potentials (IPSPs; Williams and Stuart, 2003b), whereas their deactivation reduces the duration of EPSPs. Interestingly, the forebrain-restricted deletion of the *HCN1* gene results in enhanced spatial learning in the Morris water maze task as well as improved memory of the platform location (Nolan et al., 2004).

4.3.4 Dendritic spikes

Neuronal dendrites are capable of generating large transient depolarisations called dendritic spikes that reflect the opening of voltage-gated Na^+ and Ca^{2+} channels. In some dendrites, the activation of voltage and ligand-gated NMDA receptors can result in the occurrence of an NMDA spike (Häusser et al., 2000; Larkum et al., 2009; Major et al., 2013). Dendritic spikes are typically evoked by the synchronous activation of multiple spatially clustered synapses but they can also be triggered by backpropagating APs following strong somatic stimulation (Stuart et al., 1997b; Larkum et al., 1999; Williams and Stuart, 2000b; Larkum et al., 2007; Grewe et al., 2010).

Dendritic spikes play an important role in sensory processing as well as in the timed integration of synaptic inputs (Helmchen et al., 1999; Lavzin et al., 2012; Breton and Stuart, 2009; Xu et al., 2012). They are also subject to activity-induced intrinsic plasticity. For example, in CA1 pyramidal neurons dendritic spikes are inhibited in local dendritic branches following supralinear synaptic input in that dendritic region. If the input is strong enough to trigger axonal APs, then the resulting backpropagating AP can cause a widespread attenuation of subsequent dendritic spikes in all dendritic branches (Remy et al., 2009).

4.4. Intrinsic plasticity in learning and memory

4.4.1 Invertebrate studies

The first studies suggesting a link between behavioural learning and intrinsic plasticity came from invertebrate preparations. In 1982 Alkon et al. showed this by performing voltage-clamp recordings in a nudibranch mollusk *Hermissenda* following a phototaxic learning protocol. In normal conditions, *Hermissenda* displays the tendency to move towards light. However, pairing light with rotation results in a conditioned response (CR) that is a reduction or elimination of positive phototaxis (Alkon et al., 1982). Alkon and colleagues showed that the acquisition of the CR was manifested by an increased number of AP fired by the photoreceptor cell in response to either light stimulation or direct somatic current injections. This intrinsic plasticity was mediated by a decrease in the transient A-type and Ca^{2+} sensitive K^+ currents (Alkon et al., 1984; 1985). Importantly, these changes were still present even after the photoreceptor cell was surgically isolated from neighbouring cells providing evidence that the observed changes were indeed due to a change in the cell's intrinsic properties. Similarly to the behavioural response, this increase in excitability persisted for weeks.

Later work on the terrestrial snail *Helix* also showed that associative learning significantly increased intrinsic excitability. Paired presentations of the conditioned (CS) and unconditioned stimuli (US) resulted in a lowering of the AP threshold and a positive shift in the RMP of interneurons that drive the conditioned behavioural response (Gainutdinov et al., 1998).

The marine mollusk *Aplysia* is best known for the work on the gill and siphon withdrawal reflex following non-associative (habituation, sensitisation) and associative forms of learning. In the associative conditioning task, a brief siphon tap is paired with an electrical shock to the tail. This results in a CR of withdrawing the siphon. Learning results in synaptic strengthening between the sensory and the motor neuron. Interestingly, however, learning

induced plasticity is not limited to synaptic modifications. A siphon tap or direct current injections result in an increase in the neuronal input resistance as well as in the number of APs fired by the sensory neurons (Antonov et al., 2001).

Non-associative learning such as sensitisation can also induce intrinsic plasticity. A tail-induced sensitisation of the siphon withdrawal reflex is related to increased AP firing and afterdepolarisation in sensory neurons. Sensitised motor neurons display more negative membrane potentials and a reduction in AP threshold (Cleary et al., 1998).

4.4.2 Vertebrate studies

The first vertebrate studies to provide evidence for behaviourally induced changes in neuronal excitability also come from the 1980s. Brons and Woody (1980) were the first to show intrinsic plasticity following associative conditioning in awake cats. The behavioural learning task consisted of pairing an auditory click (CS) with a tap on the nose between the eyes (US). The CR was an eyeblink combined with a nose twitch. Intracellular recordings performed in the sensorimotor cortex revealed that less current was necessary to evoke AP firing in neurons from conditioned cats when compared to those from control cats. Importantly, this change was long lasting and restricted to a specific neuronal population – it was present only in the cells that were connected with the muscles involved in generating the CR. The observed intrinsic plasticity was still present at 28 days after conditioning and was not altered by extinction training suggesting that changes in intrinsic excitability could have a long-term functional role (Brons and Woody, 1980).

More evidence for learning induced intrinsic plasticity came from experiments on rabbits that were trained in either a delayed or trace eyelid conditioning task. In both behavioural paradigms a conditioned stimulus such as an auditory or visual stimulus (CS) is paired with an eye-blink eliciting stimulus such an air puff or a weak periorbital shock. In trace eyelid conditioning the CS terminates before the delivery of the US. In delayed conditioning, on the other hand, the CS commences before the US but terminates at the same time as the US. Both learning paradigms are cerebellum dependent, but trace eyelid conditioning also requires the involvement of the hippocampus (Thompson et al., 2000).

The memory for eyeblink conditioning is long lasting and can persist from weeks to months and so can the associated plasticity of intrinsic excitability. For example, delayed eyelid conditioning performed on rabbits results in the lowering of the threshold necessary for evoking dendritic spikes as well as a notable decrease in the transient AHP. Interestingly,

these changes are spatially limited to defined microzones and the lower threshold for dendritic spike initiation is still present one month after training (Schreurs et al., 1997; 1998).

Disterhoft and colleagues found that when rabbits underwent trace eyelid conditioning, the pyramidal neurons in hippocampal regions CA1 and CA3 fired more APs in response to depolarising current injections when compared to those from control animals. Also, the post-burst AHP was significantly reduced. These changes were not present in the granule cells of the dentate gyrus and were specific only to animals that had received paired CS-US presentations and had successfully learned the task (de Jonge et al., 1990; Moyer Jr et al., 1996; Thompson et al., 1996). Increased intrinsic excitability occurred in approximately 50% of CA1 and CA3 neurons, it was visible from as early as one hour after conditioning and peaked at 24 hours. Excitability remained significantly increased for five days and then decreased considerably by day seven. Two weeks later the responses of neurons from conditioned and control rabbits were indistinguishable. The time course of the observed changes in intrinsic excitability could suggest that intrinsic plasticity might play a permissive role in memory consolidation (Moyer Jr et al., 1996; Thompson et al., 1996).

Reductions in the post-burst AHP and increased neuronal firing in CA1 neurons have also been shown to occur in the rat hippocampus following trace eyelid conditioning (Kuo et al., 2008; Oh et al., 2009) or spatial learning in the Morris water maze (Oh et al., 2003) as well as in the piriform cortex following operant conditioning (Saar et al., 1998). Operant conditioning is a behavioural training task where an animal learns to associate a stimulus with a reward or punishment. When rats learn to associate a specific odour with a water reward this results in increased neuronal excitability marked by a reduction in post-burst AHP and reduced AP accommodation. This change persists for one to three days following training and is no longer present after seven days (Saar et al., 1998).

One channel underlying the post-burst AHP is the apamin sensitive SK channel (Stocker et al., 2004). Direct infusions of the SK channel activator NS309 into the dorsal CA1 reduce spontaneous neuronal firing rates and slow down the acquisition of trace eyelid conditioning. This indicates that the AHP is an important correlate of learning due to the role it plays in regulating neuronal firing rates (Disterhoft and Oh, 2006; McKay et al., 2012).

4.4.3 Intrinsic excitability and fear conditioning

Even though changes in intrinsic excitability have been extensively demonstrated across various behavioural tasks (Disterhoft and Oh, 2006) the first examples of intrinsic plasticity

induced by fear conditioning were not provided until more recently. McKay et al., (2009) were among the first to show that both contextual and trace-auditory fear conditioning increased the excitability of CA1 hippocampal neurons through a reduction of the post-burst AHP and an increase in neuronal firing rates (McKay et al., 2009).

Intrinsic plasticity following fear learning was also shown to occur in the amygdala. Here, auditory fear conditioning resulted in a significant reduction in the post-burst sAHP of LA neurons. This change was evident immediately after training as well as 24 hours later. Fear conditioning also caused an increase in neuronal firing rates. This change however had a different time course to the sAHP as it was present 24 hours but not 1 hour after conditioning (Sehgal et al., 2014). Increased intrinsic excitability following auditory fear conditioning was also demonstrated in the basal nuclei (BA) of the amygdala where the neurons projecting to the medial prefrontal cortex (mPFC) and the hippocampus are mainly located (Hoover and Vertes, 2007). Senn et al., (2014) used retrograde labelling to identify and compare the intrinsic properties of two distinct BA neuronal populations – those projecting to the prelimbic cortex (PL) and those projecting to the infralimbic cortex (IL). The two mPFC nuclei have been identified to play different roles during fear learning and memory. The PL is implicated in the expression of conditioned fear, whereas the IL mediates fear extinction learning (Sotres-Bayon and Quirk, 2010; Sierra-Mercado et al., 2011). Senn et al. (2014) demonstrated that fear conditioning, but not extinction, increases the bursting of PL-projecting neurons. In contrast, IL-projecting neurons showed increased bursting only following extinction learning. Interestingly, similar changes in neuronal bursting activity occur *in vivo* when recording from functionally defined fear and extinction neurons. Apart from increasing bursting activity, extinction training also altered the AP kinetics of IL-projecting neurons as marked by an increase in AP width. Once again, *in vivo* recordings confirmed that the width of extracellularly recorded AP also increased following fear extinction in identified extinction neurons. On the other hand, PL-projecting neurons showed no change in AP kinetics following fear extinction (Senn et al., 2014).

Most studies report an increase in intrinsic excitability following learning (Moyer et al., 1996; Saar et al., 1998; Oh et al., 2003; McKay et al., 2013). However, experience-dependent synaptic plasticity can be bidirectional involving both synaptic strengthening and weakening (Malenka and Bear, 2004; Smith et al., 2009). Because intrinsic excitability is closely coupled to synaptic plasticity there is no reason why this bidirectional mechanism could not apply to intrinsic plasticity as well. Indeed, the learning of positive and negative

reward values in an olfactory discrimination task has opposing effects on intrinsic properties of BLA neurons. When rats learn that successful odour discrimination is associated with a water reward, the BLA neurons show increased firing in response to depolarising current injections. On the other hand, odour fear conditioning, where the rats learn to associate a given odour with a footshock result in a decrease in neuronal firing (Motanis et al., 2014).

Changes in intrinsic excitability following fear conditioning have also been shown to occur in the IL. Santini et al. (2008) showed that auditory fear conditioning results in a reduced excitability of IL neurons. This was manifested by a decrease in the number of APs elicited by depolarising current injections as well as an increase in the sAHP. Interestingly, extinction learning reversed both of these changes to levels comparable to naïve animals. Moreover, extinction increased the bursting activity of IL neurons and reduced the amplitude of the fAHP (Santini et al., 2008). One current underlying the fAHP is the M-type K^+ current. Blocking M-type K^+ currents in slices with XE-991 increases AP firing rates and promotes bursting activity. The infusion of XE-991 into the IL before extinction training reduces freezing both during the extinction learning session and during the extinction memory test performed 24 hours later. In contrast, the infusion of flupirtine, the M-type K^+ channel agonist increases freezing levels during extinction. On the other hand, when flupirtine is infused into the PL, this results in reduced fear expression while having no effect on extinction learning or recall (Santini and Porter, 2010). M-type K^+ currents are also blocked by the activation of muscarinic receptors. The infusion a muscarinic receptor antagonist into the IL impairs the recall of extinction memory, whereas the pharmacological stimulation of these receptors enhances fear extinction memory (Santini et al., 2012).

In conclusion, despite an increasing amount of research on learning-induced changes in intrinsic excitability, the precise cellular mechanisms underlying fear memory acquisition and expression in the mPFC are still largely unknown. For example, does neuronal intrinsic excitability in the PL change with learning? If so, are these changes specific to certain neuronal populations or are they more generalised? The mPFC contains a heterogenous population of neurons whose long-range connectivity might determine the way in which these neurons are altered by learning (Dembrow et al., 2010). In order to address the above questions we have formulated our research objectives, which are presented in Chapter 1 – Introduction.

5. Materials and Methods

5.1 Animals

The mice used in all of the experiments were C57Bl/6J mice obtained from Janvier Labs, France. Throughout all of the experiments, the mice were group-housed in transparent Plexiglas cages and kept in a temperature-regulated room on a 12 h light/dark schedule with free access to food and water. All the experiments were conducted in the light phase of the cycle and the mice were at least 8 weeks old at the time of any experimental manipulation. All experiments were performed in accordance with the European and French Directive 2010/63/EU (Authorisation number 5012025-A).

5.2 Stereotaxic surgery and retrograde labelling

Anaesthesia and preparation for surgery

Before the start of the surgery, the mice were anaesthetised by inhalation of isoflurane. For this purpose the mice were placed in a small Plexiglas chamber connected to tubing that delivered a mix of air and isoflurane (4% concentration) at a flow rate of 2 l/min. Once asleep the mice were transferred to a heating pad, where they continued to receive the anaesthesia through a mask. Once in the mask, the concentration of isoflurane was lowered to 2% and the flow rate was decreased to 0.4 l/min. The fur on the head was then shaved using an electrical razor. Next, the mice were injected with 0.1 µl of Lurocaïne (Vétoquinol) just under the scalp to provide local anaesthesia. Only after the above procedure was completed were the mice moved onto the stereotaxic frame (David Kopf Instruments).

Stereotaxic surgery

The mice were placed on a heating pad (HP-1M, Physitemp Instruments Inc), equipped in a rectal probe (MLT1404, Physitemp Instruments Inc) connected to a controller (TCAT-2LV, Physitemp Instruments Inc) set to maintain the body temperature at 37°C. The nose was positioned in a mask that continuously delivered a mix of isoflurane (1.5-2%) and air at a flow rate of 0.2-0.4 l/min. Once the ear bars were positioned correctly and the mouse's head was fixed in the stereotaxic frame, an eye cream (Lacrigel, Europhta) was applied to prevent the drying of the eyes. After having made sure that the mouse is properly anaesthetised (slower but stable breathing rate, absence of reflexes or signs of pain, muscle relaxation), a small incision along the medial axis was made on the scalp to expose the scull. The skin was

held open using clamps. Once exposed, the skull was cleaned and kept hydrated with NaCl (0.9%) throughout the whole surgery. Next, the head was levelled to ensure that the bregma and lambda were in the same horizontal plane. Then, the coordinates of the bregma site were identified and the injection site was located relative to the bregma point using the stereotaxic coordinates from the Paxinos & Franklin (2001) mouse brain atlas. Once the correct location was identified, the skull was thinned with a micro drill (FST) and a small needle (26 gauge, Terumo) was used to gently perforate the skull. Next, retrograde tracers Cholera toxin subunit B (CtB) Alexa Fluor conjugates 488 (CtB 488; C22841, Molecular Probes) and 594 (CtB 594; C-22842, Molecular Probles) were infused either into the contralateral medial prefrontal cortex (mPFC; 1.94 mm anterior to bregma, 0.30-0.50 lateral to bregma, 2.50 mm ventral to bregma) and/or into the ipsilateral (left) basolateral amygdala (BLA; 1.46 mm posterior to bregma, 3.40 mm lateral to bregma, 5.00 ventral to bregma). The tracers were infused at the rate of 50 nl/min. After the infusion of 100-200 nl, the infusion rate was reduced to 5 nl/min for 10 min after which the injection needle was removed. Tracer infusions were performed using a Nanofil 33 or 34 gauge bevelled needle (NF33BV-2, NF34BV-2; World Precision Instruments) attached to a 10 µl NanoFil microsyringe (NanoFil, World Precision Instruments). The microsyringe was driven by an electronic micro pump system (UltraMicroPump, World Precision Instruments) connected to a microprocessor controller (Micro4, MicroSyringe Pump Controller). Once the infusions were complete the scull was closed and the skin sutured (Ethilon 5-0, FS-3 Needle, F2413, Ethicon). The skin was treated locally with Betadine (10%, MedaPharma) for local disinfection. The mice were then rehydrated through intraperitoneal injections of 0.2-0.3 ml of saline. The mice were left to recover on a heating pad. Once awake they were injected subcutaneously with an analgesic Buprenorphine (0.01-0.05 mg/kg, Axience). The mice were allowed to recover for one week before any further experimentation.

5.3 Contextual fear conditioning

Apparatus

The mice were fear conditioned in a grey Perspex chamber (length 26 cm, width 18 cm, height: 25 cm, Imetronic, Pessac, France) with a metal grid floor that could be electrified to deliver a mild scrambled electric shock. The chamber was located inside a sound-attenuating box (length: 55 cm, width: 60 cm, height: 50 cm; Imetronic, Pessac, France) and was illuminated by four small overhead lights. A video camera placed above the conditioning box

allowed for the observation and the recording of the animals' behaviour. The mice were tested for memory recall either in the same chamber in which they had been conditioned (SAME) or in a novel context (NOVEL). The NOVEL context was a round cylinder (diameter 20 cm, height 27 cm) with white and grey diagonal stripes. The metal grid floor was replaced with a plastic floor tray covered with sawdust.

Behavioural procedure

The mice were divided into three behavioural groups: conditioned (COND), context only (CTX) and immediate shock (IMM). All mice were handled for five days before the onset of training. On the day of the training the mice were placed in the fear-conditioning chamber for a 5 min long behavioural session. The COND mice were allowed to explore the context for 2 min after which they received three unsignalled footshocks (1 s in duration, 0.5 mA). Each footshock was separated by a 1 min interval. After the delivery of the last shock the COND mice remained in the conditioning chamber for an additional minute before being removed and placed back in their home cage. The CTX mice were also placed in the conditioning chamber but in contrast to the COND mice they did not receive any footshocks and were allowed to freely explore the context for the entire 5 min. The IMM mice received three unsignalled footshocks (1 s in duration, 0.5 mA) immediately upon placement in the conditioning context. Each shock was separated by a 1 s interval. After the delivery of the last shock, the IMM mice remained in the conditioning chamber for the remaining 5 min before being placed back in their home cages. The COND group constituted the learning group whereas the CTX and IMM mice were used as control groups. Between mice, floor trays and shock bars were cleaned with ETOH 70%.

Fear memory was tested 24 h following training. During the recall phase mice were placed back in the conditioning chamber for 3 min, after which they were returned back to their home cage. All behavioural groups underwent the same memory recall procedure. Freezing was analysed manually using a custom written analysis programme BehavScor (version 3.0 beta, 2008, by A. Dubreucq). Freezing was classified as complete lack of movement apart from breathing (Fanselow, 2000). Every time freezing was observed the experimenter pressed and held down a key on the keyboard. The key was released when the mouse moved again. The time spent freezing was then calculated as a percentage of the total time spent in the chamber.

In order to control for the specificity of the memory, the COND and CTX mice were also tested for memory retrieval 7 days following training. Here, the memory test was performed either in the SAME or in the NOVEL context. As before, the memory retrieval session was 3 min long. In the NOVEL context the sawdust was exchanged between each mouse.

The mice that had undergone stereotaxic surgery and were used for electrophysiological recordings were never tested for memory retrieval. However, a subset of mice was tested regularly for memory recall in order to ensure that no change in the efficacy of the training protocol had taken place. These mice were not used for electrophysiological recordings.

5.4 Electrophysiology

Cortical slice preparation

The mice were sacrificed for electrophysiological recordings 1-4 days following the behavioural session. The mice were anaesthetised with isoflurane and intercardiacally perfused with ice-cold (4°C) artificial cerebrospinal fluid (aCSF) consisting of the following (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 1 $CaCl_2$, 7 $MgCl_2$, 7 D-glucose, 3 kynurenic acid, 200 sucrose, 1.3 ascorbic acid, and 3 sodium pyruvate. The solution was bubbled with 95% O_2 and 5% CO_2 to maintain a pH of ~ 7.4. Following the perfusion, the mice were decapitated, the heads were immersed in ice-cold aCSF and the brains were removed rapidly. Next, the frontal cortex was isolated by making a coronal cut approximately at bregma point to maximise the dendritic projections within the plane of the slice. The front half of the brain was then glued to the cutting surface of the vibrating tissue slicer (Vibratome 3000 Plus, Sectioning Systems), rostral end up with the dorsal side facing the blade. The second half of the brain, containing the BLA, was preserved and fixed overnight in 4% PFA at 4°C in order to verify the accuracy of the stereotaxic injection. The accuracy of the mPFC injection was readily observable during the electrophysiological experiment. Coronal slices of 300 μm were cut and gently transferred using a pasteur pipette with a custom made open end into an incubating chamber filled with aCSF containing (in mM): 100 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 1 $CaCl_2$, 7 $MgCl_2$, 7 d-glucose, 3 kynurenic acid, 1.3 ascorbic acid, and 3 sodium pyruvate bubbled with 95% O_2 and 5% CO_2. Following 15-20 min of incubation at 37°C slices were transferred to a second incubation chamber containing (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, 10 d-glucose, 1.3 ascorbic acid, and

3 sodium pyruvate bubbled with 95% O_2 and 5% CO_2. The slices were left to incubate for another 20 min at 37°C after which they were stored at room temperature (22°C) until the time of recording. All recordings were performed blind with respect to the behavioural group assignment.

The part of the brain that was preserved after slicing and fixed overnight in 4% PFA was rinsed 3 times in 0.1 M PB. Coronal slices of 100 μm were cut using a vibrating tissue slicer (Leica VT 1000S) and next visualised under standard inverted microscope (Zeiss Axiovert 100) equipped in 470 nm/525 nm and 550 nm/605nm excitation/emission filter sets to verify the accuracy of the BLA injections. Recordings from neurons where the injections were inaccurate or not limited to the BLA were excluded from the analysis.

Whole-cell recordings

Whole-cell current-clamp recordings were performed on slices submerged in a chamber filled with aCSF heated to 32 -34°C via a Scientifica system equipped in a heated perfusion tube (HPT-2, ALA Scientific Instruments). The slices were continuously superfused with aCSF bubbled with a mixture of 95% O_2 and 5% CO_2. The flow rate was set at 1-2 ml/min to ensure sufficient oxygenation but also to minimise mechanical disruption of the recordings. The aCSF was identical to that used during the second incubation. Neurons were visualised using a standard upright microscope (Carl Zeiss Axio Examiner.D1) using the Dodt contrast method (Dodt & Zieglgänsberger, 1990), under a 63x water-immersion objective (Zeiss). CtB-labelled neurons were excited via a Compact Light Source HXP 120 (Leistungselektronic JENA GmbH) filtered through 485 nm/535 nm and 560 nm/645 nm excitation/emission filters for CtB 488 and for CtB 594 respectively. Neurons were visualised using an Evolve 512 EMCCD Camera (Photometrics). Patch pipettes (4-7 MΩ) were pulled from capillary glass, dimensions: 1.16 x 2.00 x 80.00 mm. (Science Products) using a Flaming/Brown micropipette puller (Model P-97, Sutter Instruments). The pipettes were then filled with internal solution containing the following (in mM): 135 K-gluconate, 10 HEPES, 10 Na_2-Phosphocreatine, 4 KCl, 4 Mg-ATP and 0.3 Na-GTP. Data were acquired using a Dagan BVC-700A amplifier and AxoGraph X (version 1.3.5). Recordings were filtered at 3 kHz and digitised at 20 kHz using an ITC-16 (InstruTech). All recordings were performed in the presence of fast synaptic activity blockers: 50μM of DL-AP5 (DL-2-Amino-5-phosphonopentanoic acid sodium salt; #ab120271, Abcam), 3μM of NBQX (2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide disodium salt; #ab120046, Abcam), and 10μM

of gabazine (2-(3-Carboxypropyl)-3-amino-6-(4 methoxyphenyl)pyridazinium bromide; SR95531; #ab120042, Abcam). All drugs were made up as 1000x stock solutions in distilled water and were diluted to the desired concentration in aCSF at the start of the experiment. Drugs were allowed 10 min to wash in before taking any measurements. The pipette capacitance was compensated and the bridge was balanced before each recording. Series resistance was measured throughout the experiment. The experiment was terminated if the series resistance was larger than 30 MΩ. Biocytin (Sigma) 1.5-2.5 mg/ml was included in the internal solution for post-hoc morphological identification of the recorded neurons. At the end of the electrophysiological recordings the slices were fixed overnight in 4% paraformaldehyde (PFA) in phosphate buffered saline (PB), pH 7.4. The following day the slices were washed 3 times for 10 min in 0.1 M PB and stored in 0.1M PB at 4°C for up to 2 months.

5.5 Biocytin labelling procedure

Biocytin labelling was performed on fixed free-floating 300 µm slices that had been used for electrophysiological recordings. Slices were processed in a 24 well plate each slice occupying one well. Slices were first washed 3 times in 0.1 M PB for 10 min after which they were incubated for 15-30 min in a freshly prepared H_2O_2 solution (3% H_2O_2 in 0.1 M PB) to block any endogenous peroxidase activity. This incubation resulted in the development of oxygen bubbles. Next, the slices were washed 5 times with 0.1 M PB for 10 min. The slices were then permeabilised for 1 h in a 0.1 M PB solution containing 2% Triton X-100 (wt/vol Sigma; e.g 0.4 g Triton X-100 in 20 ml 0.1 M PB). Next, the slices were incubated for at least 2 h at room temperature in the ABC solution (Vectastain Elite kit, avidin-biotin complex, Vector Laboratories, PK-6100) in 0.1 M PB containing 1% Triton X-100 (e.g. 4 drops of #A, 4 drops of #B and 0.2 g of Triton X-100 in 20 ml of 0.1 M PB). Following the incubation, the slices were washed 5 times for 10 min with 0.1 M PB. The chromagenic reaction allowing for biocytin visualisation was started by placing the slices in a solution containing 3,3'-Diaminobenzidine Tetrahydrochloride (DAB; # D-5637 Sigma; taken out of a stock solution of 17.5 mg/ml) and 0.01% H_2O_2 in 0.1 M PB. The slices were incubated in this solution for several minutes and the reaction was observed under a dissection microscope. When the desired contrast between the cell and the slice was reached, the reaction was stopped by transferring the slices into 0.1 M PB. After that the slices were washed 5 times for 10 min.

DAB-processed slices were mounted using polyvinyl alcohol 4-88 (Mowiol 4-88) mounting medium (Fluka #81381) and visualised with a brightfield microscope. Neurons that were not located in the PL or did not have the appearance of pyramidal-shaped neurons were excluded from the analysis. Example neurons were reconstructed using a 100x oil-immersion objective with a computer-controlled system running Neurolucida imaging software (MicroBrightField Bioscience).

5.6 Data analysis

All data were acquired and analysed using AxoGraph X. The resting membrane potential (RMP) was recorded after break-in after having allowed several minutes for the cell to stabilise. To examine the effects of behavioural training on membrane excitability, PL neurons were injected with 800 ms current pulses ranging from -200 to 300 pA at 20 pA increments. The input resistance was measured as the slope of the linear fit of the voltage-current plot between -100 pA and -40 pA. The rheobase was defined as the minimum amount of current necessary to evoke the first action potential (AP). The first AP in a train of 4-5 AP was analysed for AP threshold, maximum rate of rise (*dV/dt* in mV/ms), AP amplitude and AP half-width. Threshold was determined at the point when the *dV/dt* exceeded 10 mV/ms. AP amplitude was measured from threshold to peak and the half-width was measured at half this distance. The interspike interval (ISI) was measured between the first two APs in a train of 4-5 APs. The number of APs evoked by depolarising current steps as well as the maximum number of APs fired was also measured. The resonance frequency was measured using sinusoidal current injections of constant amplitude (30-100 pA: adapted to prevent AP firing; average of 3 repetitions) and linearly increasing frequency 0-20 Hz in 20 s. The impedance amplitude profile (ZAP) was determined by taking the ratio of the fast Fourier transform of the voltage response to the fast Fourier transform of the current injection (Ulrich, 2002; Narayanan and Johnston, 2007). The ZAP plot was then fitted with 5-7 exponential fits and the resonance frequency was defined as the peak of the exponential fit. The sag response was measured using hyperpolarising current injections (-100 pA, 800 ms, average of 10 repetitions). In order to calculate the sag, two values were measured: the voltage difference between the RMP and the peak of the hyperpolarisation as well as the voltage difference between the steady-state voltage and the peak of the hyperpolarisation. The sag ratio was then calculated as the ratio of the two values and expressed as a percentage. The membrane time constant was measured through the injection of alternating depolarising and hyperpolarising

current pulses (400 pA, 1 ms, 10 repetitions). It was then calculated as the slow component of a double-exponential fit of the average voltage decay in both the depolarising and hyperpolarising directions. The medium afterhyperpolarisation (mAHP) was measured as the maximum negative peak following the last AP in a train of 5 AP evoked at frequencies ranging from 20 to100 Hz (at 20 Hz intervals) by 2 ms current pulses of 2 nA. The slow AHP (sAHP) was measured as the maximum negative peak following the last AP in a train of 15 AP at 50 Hz evoked by 2 ms current pulses of 2 nA.

Statistical analyses were performed using the unpaired student's *t*-test, a one-way ANOVA or a two-way mixed-design ANOVA. After a significant main effect, post hoc comparisons were performed with the Tukey test, Bonferroni's multiple comparisons test or Dunnett's multiple comparisons test. The group size, mean and the standard error of the mean (SEM), as well as the exact statistical test used for each analysis are provided in the corresponding figure legends in the Chapter 6 - Results.

6. Results

6.1 Behavioural training

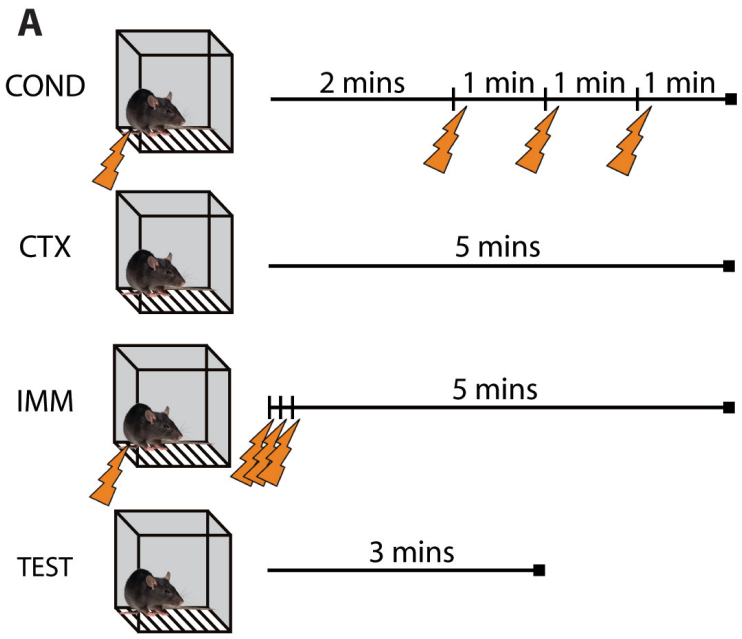
In order to investigate how learning alters the excitability of prelimbic (PL) neurons we first established a reliable contextual fear conditioning protocol. Fear conditioning is a useful behavioural model in which learning is rapid and long lasting (Fanselow, 1990; Anagnostaras et al., 2000; Gale et al., 2004), with a single training session being sufficient to induce changes in neuronal excitability (McKay et al., 2009). However, in the case of contextual fear conditioning these changes in excitability can also be easily reversed by contextual re-exposure that occurs during memory retrieval. Re-exposing the animal to the training context in the absence of an electric shock acts as a single extinction trial. It reduces freezing responses and reverses learning induced changes in excitability to levels observed in naïve animals (McKay et al., 2009). In order to bypass this problem we performed all of the electrophysiological recordings on mice that were conditioned but that had not undergone memory retrieval. For this reason, it was very important to first establish a behavioural protocol where the mice showed robust learning and where the overlap between freezing scores from trained and control animals was minimal. In order to find the best parameters we tested a combination of different footshock intensities (0.5 mA, 0.7 mA), durations (1 s, 2 s) and repetitions (1, 3). After these tests we selected the following protocol because it gave the best and most consistent results: a repetition of three shocks 1 s in duration at 0.5 mA. The results of this protocol are presented below.

The mice were trained and tested in three behavioural groups: a conditioned group (COND), and two control groups, context only (CTX) and immediate shock (IMM). COND mice received three unsignalled footshocks (separated by 1 min intervals) preceded by 2 min of context exploration. In contrast, the CTX mice were exposed to the training context but received no footshocks, whereas the IMM mice received three consecutive footshocks (at 1 s intervals) immediately upon placement in the conditioning context. The length of the behavioural session was the same for each behavioural group and lasted 5 min (Figure 6.1*A*). To test the difference in freezing levels between the three behavioural groups we re-exposed a subset of mice to the training context 24 h after conditioning. The analysis of the average freezing responses of COND, CTX and IMM mice revealed that COND mice showed robust learning by freezing significantly more than both the CTX ($p < 0.001$) and the IMM mice ($p < 0.001$; Figure 6.1*B*). Importantly, the cumulative distribution plot presented in Figure 6.1*C*

indicates that 70% of the COND mice froze more than 40% of the time, while none of the CTX or the IMM mice did. Moreover, 80% of the mice in the CTX and IMM groups froze less than 20% of the time. These results confirmed that the established fear conditioning protocol was robust and specific to the learning group as the overlap between scores of the COND mice and the two control groups was minimal.

Contextual fear memory is known to remain specific to the conditioning context for at least 14 days following training. Afterwards, the memory becomes less detailed and more generalised as it undergoes consolidation in the prefrontal cortex (Wiltgen & Silva, 2007). In our experiments the mice were sacrificed for electrophysiological recordings 1-4 days following the behavioural session, at a time when the memory for the context should still be detailed. However, to ensure that with our protocol the contextual fear memory also remains specific for at least one week following training, we tested a group of COND mice for memory retrieval either in the same context in which they had been conditioned (SAME) or in a novel one (NOVEL, Figure 6.1*D*). Figure 6.1*E* shows that the mice that were re-exposed to the SAME context froze significantly more than those tested in the NOVEL context (p = 0.0045). These results confirm that in our behavioural protocol the memory for the context remains specific for at least 1 week following training.

Figure 6.1 Contextual fear conditioning – experimental design. ***A***, Three experimental groups were studied. In the conditioned group (COND), mice explored the context for 2 min after which they received 3 unsignalled footshocks (each 1 min apart). In the control group (CTX) mice explored the context without receiving any footshocks. In the immediate shock group (IMM) mice received three consecutive unsignalled footshocks immediately upon placement in the context. All behavioural sessions lasted 5 min. The memory was tested 24 h after conditioning for 3 min. ***B*,** Mean percentage of freezing across all behavioural groups. As expected, COND mice (45.61 ± 4.48%, n = 10) froze significantly more than CTX (10.96 ± 2.81%, n = 10, $p < 0.001$) and IMM mice (14.15 ± 3.23%, n = 10 $p < 0.001$; $F_{2,25} = 28.80$, $p < 0.0001$). ***C,*** The cumulative percentages showing differences in freezing levels between the COND group and the two control groups: CTX and IMM ***D,*** To validate that the memory for the context was specific during the first post-conditioning week, COND mice were tested 7 days after training either in the conditioning context (SAME) or in a novel context (NOVEL). ***E,*** Mean percentage of freezing for COND mice during tests in the SAME and NOVEL environment. COND mice tested in the NOVEL environment (11.95 ± 4.57%, n = 8) froze significantly less than those exposed to the SAME context (36.85 ± 5.66%, n = 8, $t(14) = 3.38$, p = 0.0045). Data are shown as the mean ± SEM, ***$p < 0.001$, **$p < 0.01$. Statistical significance was calculated using a one-way ANOVA with a Tukey post-hoc test (***B***) or an unpaired student's *t*-test (***E***).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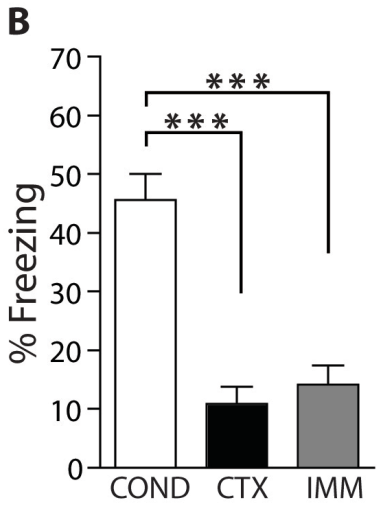
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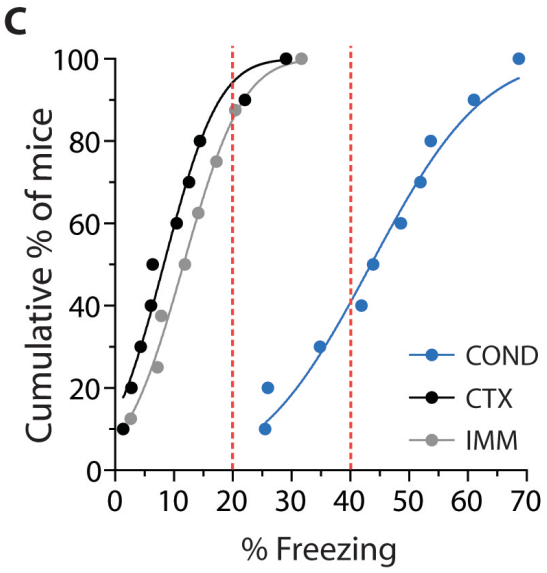
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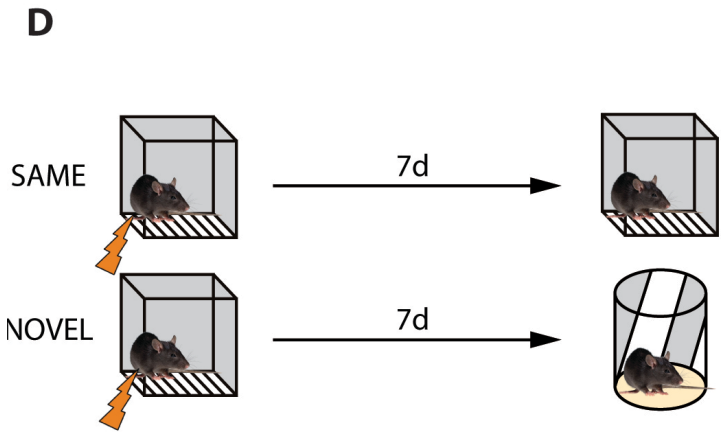
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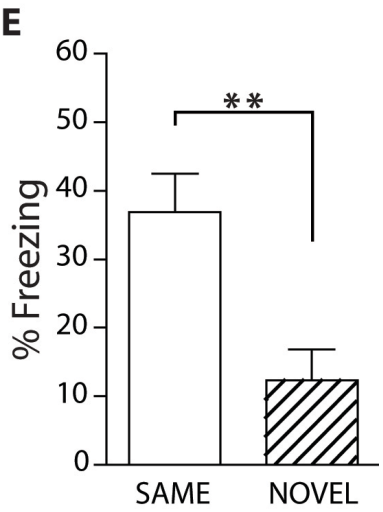
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6.2 Subthreshold properties of the BLA- and mPFC-projecting PL neurons following fear conditioning

The first objective of this project was to investigate whether contextual fear conditioning induces changes in the intrinsic excitability of BLA- and mPFC-projecting PL neurons. We selected the mPFC-projecting group because this pathway could be important for mediating responses to stressful situations (Lupinsky et al., 2010) and the BLA-projecting group because of this pathway's importance in fear memory acquisition and expression (Burgos-Robles et al., 2009; Orsini et al., 2011; Stevenson 2011; Vouimba and Maroun, 2011). In order to identify the two neuronal groups of interest a retrograde tracer Cholera toxin B (CtB) was infused into the ipsilateral BLA and/or the contralateral mPFC (Figure 6.2*A,B*). The neurons from both projection groups were distributed throughout the superficial layers 2-3 and the deeper layers 5-6 in agreement with previous research (Gabbott et al., 2005; Dembrow et al., 2010; Figure 6.2*B,* layer 6 not shown). For the purpose of this study we limited our recordings to layers 2/3 and 5. During recordings, neurons were filled with biocytin and at the end of the experiment the slices were fixed for subsequent DAB processing and neuronal identification. Biocytin labelled neurons were identified as pyramidal based on their morphology. Figure 6.2*C* shows an example of a reconstructed BLA- and mPFC-projecting neuron.

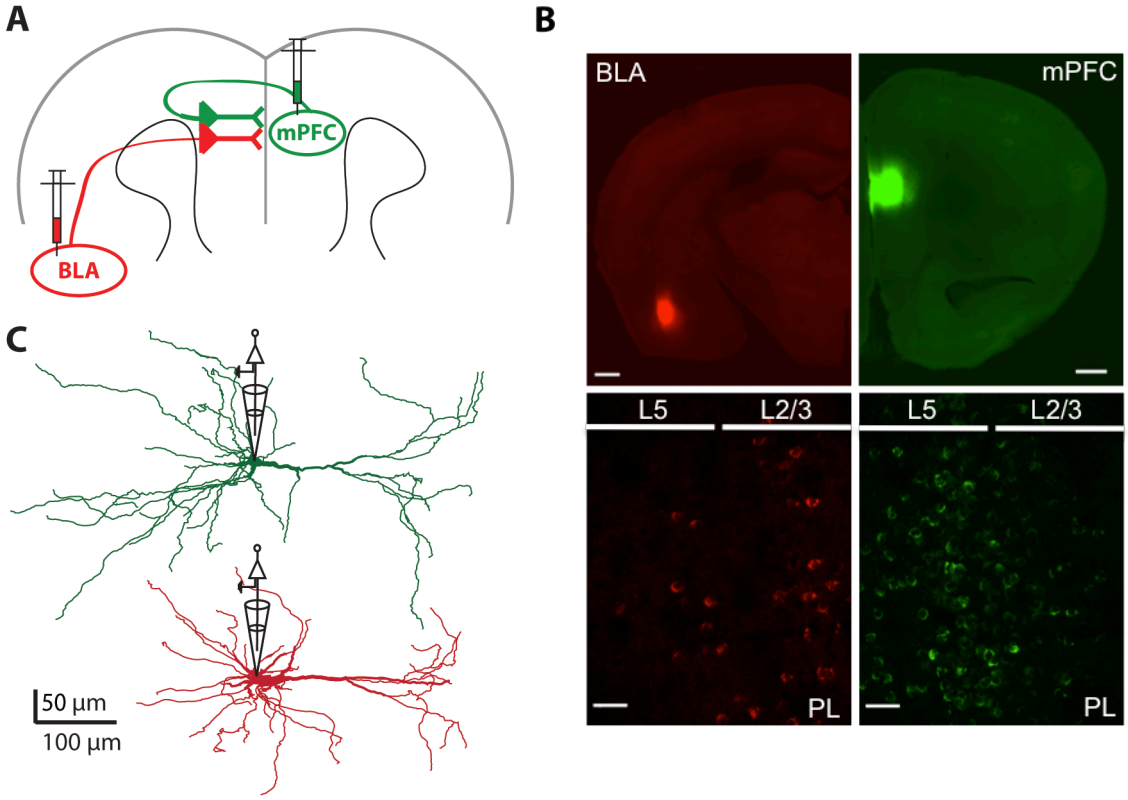

Figure 6.2 Retrograde labelling ***A,*** Schematic of stereotaxic injections strategy. Cholera toxin B (CtB), Alexa Fluor conjugates were infused into the ipsilateral BLA (Alexa 549) and/or into the ipsilateral mPFC (Alexa 488). ***B,*** Representative coronal slices displaying the site of the Ctb injections (BLA injection, top left; mPFC injection, top right) and the resulting labelling in the PL (BLA injection, bottom left; mPFC injection, bottom right). Scale bars represent 500 µm (top) 50 µm (bottom). ***C,*** Representative reconstructions from biocytin filled and DAB processed BLA- (red) and mPFC-projecting (green) neurons.

In order to investigate the effect of fear conditioning on the intrinsic excitability of the mPFC- and BLA-projecting neurons, we started by measuring their subthreshold intrinsic properties in two groups of mice: COND and CTX. This analysis revealed that the BLA-projecting neurons from COND mice had more depolarised resting membrane potentials (RMP) compared to those from CTX mice (p = 0.0016, Table 6.1). No differences between the two behavioural groups were found in the neuronal input resistance (R_N; p = 0.53), resonance frequency (f_R; p = 0.28**)**, sag ratio (p = 0.50), membrane time constant (**τ)** in both the depolarising (p = 0.68) and hyperpolarising directions (p = 0.48) and in the rheobase (p = 0.45; Table 6.1). The rheobase is defined as the minimum amount of current necessary to evoke at least one action potential (AP).

Table 6.1 Electrophysiological properties of the BLA-projecting neurons

BLA-projecting	COND (n = 14)	CTX (n = 10)
RMP (mV)	**-65 ± 0.71**[a]	**-69 ± 0.83****
R_N (mΩ)	168 ± 11.36	158 ± 9.62
f_R (Hz)	0.74 ± 0.13	0.53 ± 0.12
Sag ratio (%)	8.99 ± 1.09	7.79 ± 1.42
Membrane τ depolarising (ms)	26.03 ± 1.24	26.76 ± 1.05
Membrane τ hyperpolarising (ms)	24.94 ± 1.12	26.46 ± 1.94
Rheobase (pA)	67 ± 6.50	60 ± 5.97
AP threshold (mV)	-36 ± 0.48[a]	-38 ± 0.99
AP amplitude (mV)	81 ± 1.25	83 ± 0.95
AP max *dV/dt* (mV/ms)	**318 ± 9.06**	**346 ± 5.89***
AP half-width (ms)	**1.10 ± 0.03**	**1.00 ± 0.02***

[a]For these parameters n = 12. Values for 2 cells were excluded due to offset problems.
Data are shown as the mean ± SEM. Statistical significance was calculated with an unpaired student's *t*-test. *$p < 0.05$, **$p < 0.01$

Table 6.2 Electrophysiological properties of the mPFC-projecting neurons

mPFC	COND (n = 10)	CTX (n = 13)
RMP (mV)	-71 ± 1.59	-70 ± 1.23
R_N (mΩ)	165 ± 8.39	164 ± 9.09
f_R (Hz)	1.00 ± 0.12	1.02 ± 0.12
Sag ratio (%)	8.12 ± 0.86	6.34 ± 0.69
Membrane τ depolarising (ms)	23.81 ± 0.90	23.36 ± 1.00
Membrane τ hyperpolarising (ms)	27.19 ± 1.40	22.90 ± 1.54
Rheobase (pA)	60 ± 4.22	68 ± 5.79
AP threshold (mV)	-38 ± 0.87	-40 ± 0.55
AP amplitude (mV)	82 ± 1.18	84 ± 0.90
AP max *dV/dt* (mV/ms)	364 ± 12.34	361 ± 10.65
AP half-width (ms)	0.91 ± 0.02	0.99 ± 0.03

Data are shown as the mean ± SEM. Statistical significance was calculated with an unpaired student's *t*-test.

In contrast to the BLA-projecting neurons, the RMP of the mPFC-projecting neurons did not differ between COND and CTX mice (p = 0.73; Table 6.2). There were also no between group differences in the R_N (p = 0.98), f_R (p = 0.89**)**, sag ratio (p = 0.12), membrane τ in both the depolarising (p = 0.75) and hyperpolarising directions (p = 0.06) and in the rheobase (p = 0.32; Table 6.2). These results indicate that contextual fear conditioning causes a depolarised shift in the RMP of the BLA- but not of the mPFC-projecting neurons. However, none of the other investigated subthreshold parameters are affected by fear conditioning in either neuronal population.

6.3 Contextual fear conditioning does not alter the number of APs fired in response to depolarising current steps

Intrinsic excitability is often measured as a function of how many APs are fired by the neuron in response to fixed-duration incremental depolarising current injections (Mozzachiodi & Byrne, 2010). For this reason we applied 800 ms long pulses at 20 pA increments. We counted the number of APs elicited by each current intensity between 20 and 300 pA as well as the maximum number of APs elicited by each current step. Figure 6.3*A* shows example responses of BLA- and mPFC-projecting neurons from both COND and CTX mice to depolarising current pulses of intensities between 20 and 80 pA. The analysis of the BLA-projecting neurons revealed that fear conditioning had no effect on the number of APs fired at any of the current intensities by neurons from COND and CTX mice (p = 0.25; Figure 6.3*B*). Also, no between group differences were observed in the maximum number of APs elicited by these neurons (p = 0.22; Figure 6.3*C*).

The analysis of the mPFC-projecting neurons also did not reveal any differences in the the number of APs elicted at any current intensity (p = 0.86; Figure 6.3*B*) or in the maximum number of APs fired by neurons from COND and CTX mice (p = 0.83; Figure 6.3*C*). These results indicate that contextual fear conditioning does not alter the number of APs elicited by depolarising current steps in either the BLA- and mPFC- projecting neurons.

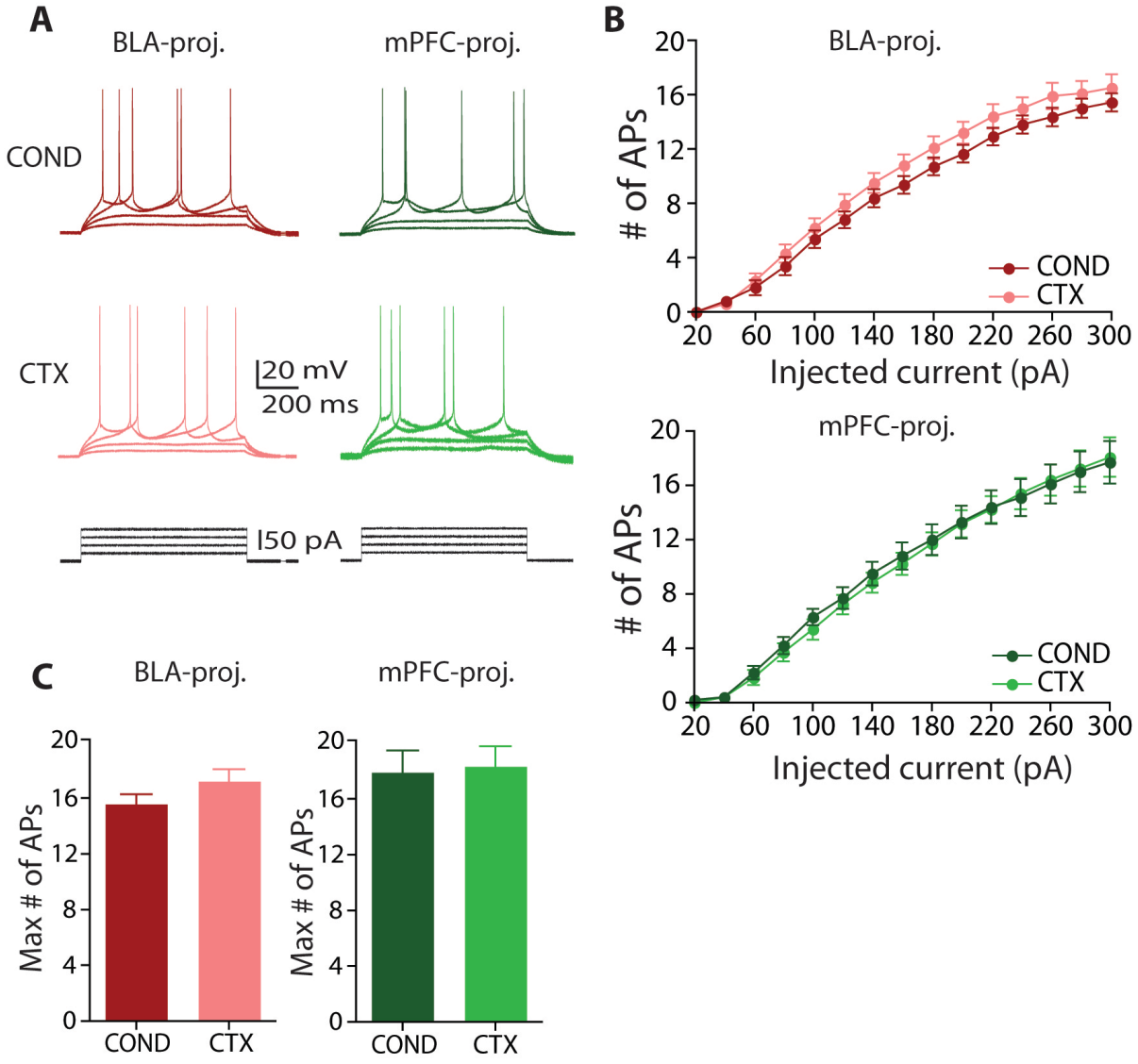

Figure 6.3 Contextual fear conditioning does not alter the number of APs fired in response to depolarising current pulses. ***A,*** Example traces from BLA (red) and mPFC-projecting (green) neurons from COND and CTX mice in response to 800 ms step current injections of 20 to 80 pA at 20 pA increments. ***B,*** Fear conditioning had no effect on the number of APs fired in response to depolarising steps of different current intensities in both BLA- (COND: $n = 14$, CTX: $n = 10$; $F_{1,308} = 1.38$, $p = 0.25$) and mPFC-projecting neurons (COND: $n = 10$, CTX: $n = 13$; $F_{1,294} = 0.03$, $p = 0.86$). ***C,*** The maximum number of APs fired did not differ between neurons from COND and CTX mice in both the BLA- (COND, 16 ± 0.72, $n = 14$; CTX, 17 ± 0.87, $n = 10$; $t(22) = 1.27$, $p = 0.22$) and the mPFC-projecting group (COND, 18 ± 1.57, $n = 10$; CTX, 18 ± 1.43, $n = 13$; $t(21) = 0.21$, $p = 0.83$). Data are shown as the mean ± SEM. Statistical significance was calculated using a two-way mixed-design ANOVA (***B***) or an unpaired student's *t*-test (***C***).

6.4 Contextual fear conditioning alters the AP kinetics of the BLA- but not of the mPFC-projecting neurons

Fast neuronal communication arises from the conversion of synaptic inputs received in the somatodendritic regions into all-or-none APs usually generated in the axon initial segment (Stuart et al., 1997b) While, APs have traditionally been perceived as binary signals that transmit information via their rate and temporal pattern, this view is now being challenged. Emerging evidence suggests that the AP waveform could be an important carrier of information (Debanne, 2004). It is now known that the APs shapes are not random but instead they reliably depend on previous stimulus history and conductance (Polavieja et al., 2005; Juusola et al., 2007).

Figure 6.4*A* shows traces of the first AP in a BLA- and mPFC- projecting neuron evoked by sufficient current to trigger four APs in 800 ms. The corresponding phase plots are presented in Figure 6.4*B*. The analysis of the AP kinetics revealed that the BLA-projecting neurons from COND mice fired APs that had a slower maximum rate of rise (dV/dt; $p = 0.026$ and a longer half-width ($p = 0.035$) than those from CTX mice (Table 6.1; Figure 6.4*BC* and Figure 6.5*A,B*). There was no difference in the AP threshold ($p = 0.11$) or amplitude ($p = 0.37$; Table 6.1) between these neurons. Because of this change in AP dynamics we were interested to find out whether there was a change in the first interspike interval (ISI). For this reason we measured the ISI between the first two APs in a train of 4-5 APs. We observed a strong but not significant trend towards an increased first ISI in COND mice when compared to CTX mice ($p = 0.0504$), suggesting that the slower AP kinetics of neurons from COND mice could prevent these neurons from firing bursts of APs (Figure 6.5*C*).

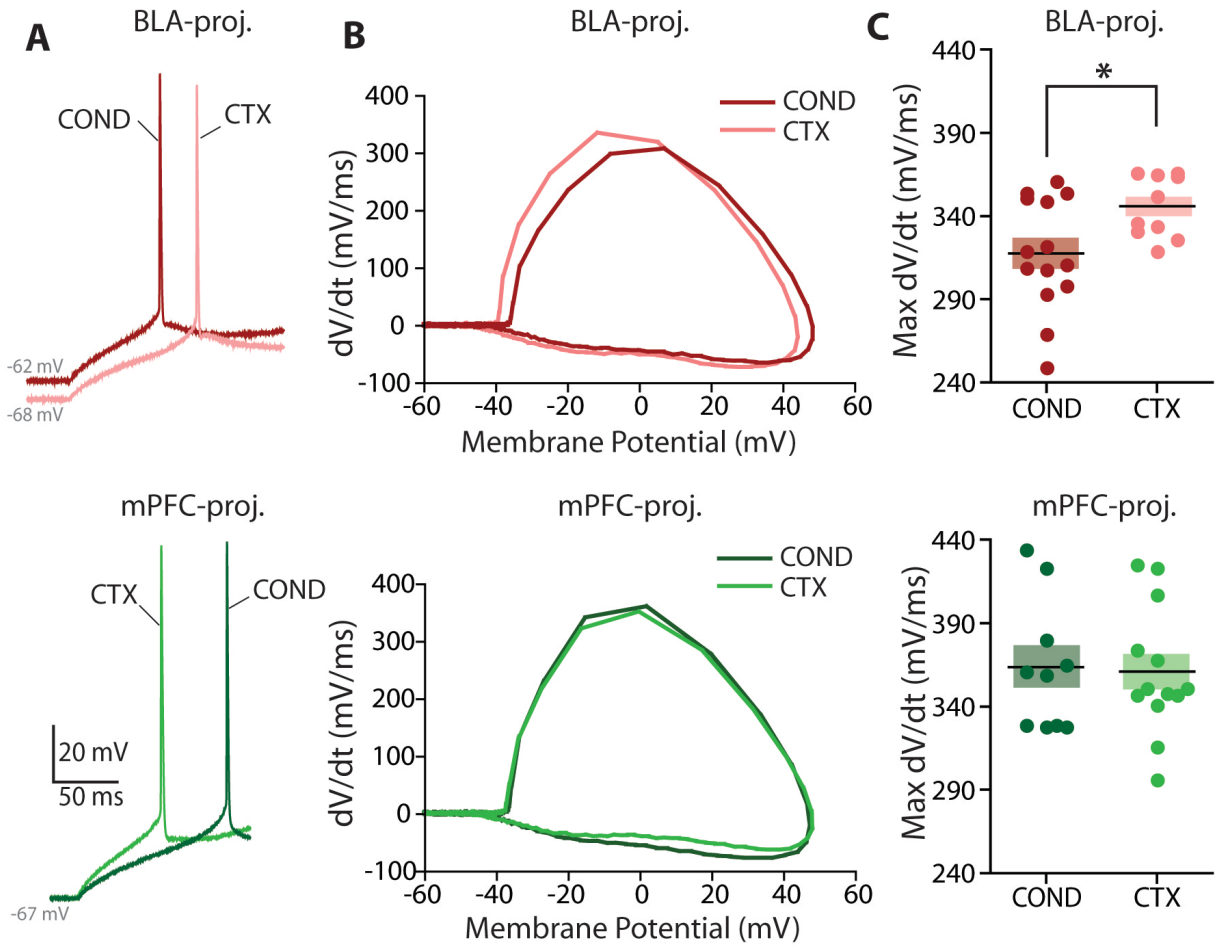

Figure 6.4 Fear conditioning induces a slower maximum *dV/dt* in the BLA- but not in the mPFC-projecting neurons *A,* Example traces showing the first AP fired by BLA (red) and mPFC-projecting (green) neurons from both COND and CTX mice in response to an 800 ms step current injection sufficient to evoke 4 APs. ***B,*** Phase plots for the corresponding APs shown in (***A***). Maximum *dV/dt* was measured as the maximum peak of the phase plot. ***C,*** Fear conditioning resulted in BLA-projecting neurons from COND mice (318 ± 9.06 mV/ms, n = 14) having a significantly slower maximum *dV/dt* than those from CTX mice (346 ± 5.89 mV/ms, n = 10; $t(22) = 2.38$, $p = 0.026$). In contrast, fear conditioning did not alter the maximum *dV/dt* of the mPFC-projecting neurons (COND, 364 ± 12.34 mV/ms, n = 10; CTX, 361.1 ± 10.65 mV/ms, n = 13, $t(21) = 0.16$, $p = 0.88$). Data are shown as the mean ± SEM. Statistical significance was calculated with an unpaired student's t-test (***C***). *$p < 0.05$.

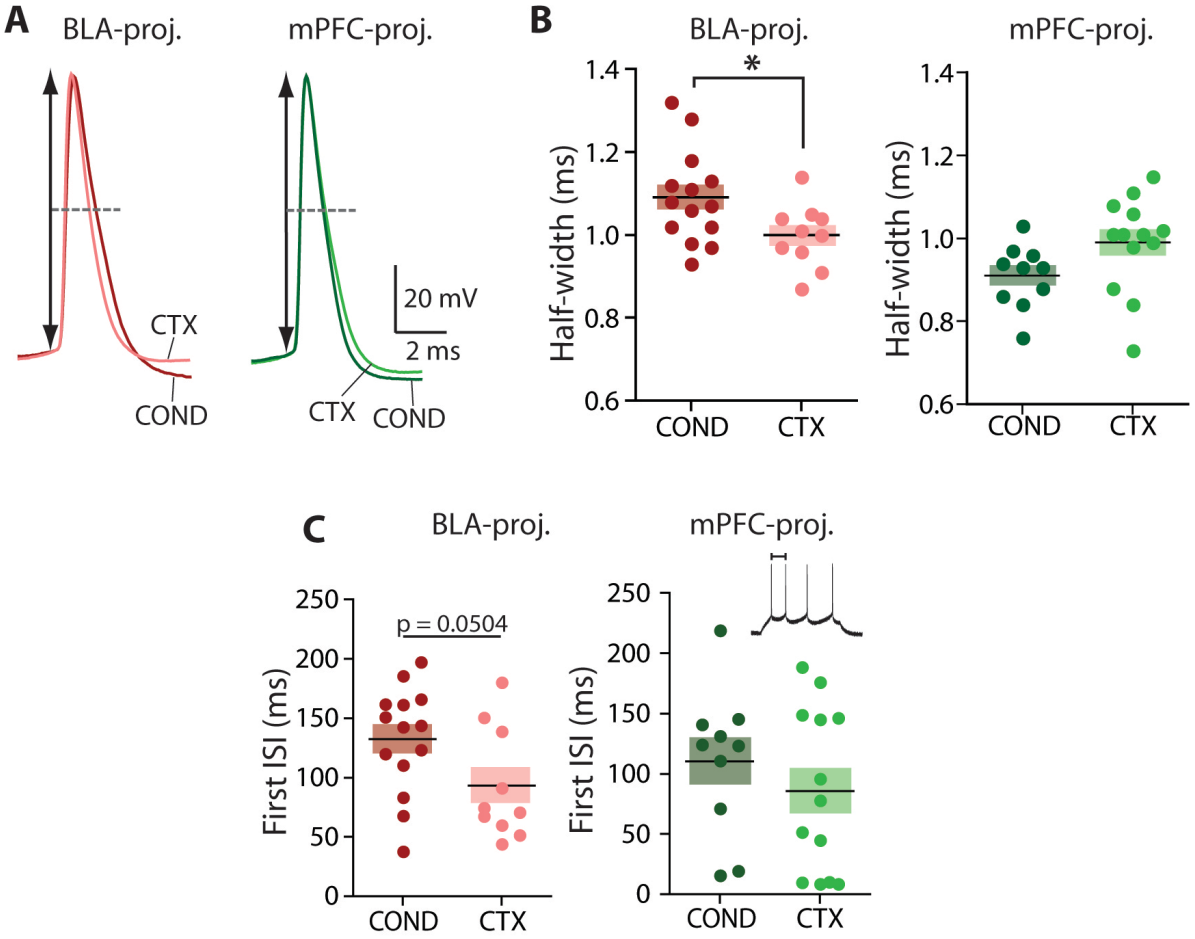

Figure 6.5 Fear conditioning increases the AP half-width in the BLA- but not in the mPFC-projecting neurons. ***A,*** Example traces showing the AP half-width measured at half the distance between the AP threshold and peak for BLA (red) and mPFC-projecting (green) neurons from both COND and CTX mice. ***B,*** BLA-projecting neurons from COND mice (1.09 ± 0.03 ms, n = 14) had increased AP half-width when compared to those from CTX mice (1.00 ± 0.02 ms, n = 10; $t(22)$ = 2.24, p = 0.035). The AP half-width of mPFC neurons from COND (0.91 ± 0.02 ms, n = 10) and CTX (0.99 ± 0.03 ms, n = 13) mice did not differ ($t(21)$ = 1.90, p = 0.071). ***C,*** In the BLA-projecting neurons fear conditioning resulted in a strong but not significant trend towards a longer first ISI in COND mice when compared to CTX mice (COND, 133 ± 12 ms, n = 14; CTX, 93 ± 15 ms, n = 10; $t(22)$ = 2.07, p = 0.0504). Fear conditioning did not alter the first ISI in the mPFC-projecting cells (COND, 110 ± 19 ms, n = 10; CTX, 86 ± 19 ms, n = 13; $t(21)$ = 0.90, p = 0.38). The inset is an example trace showing the ISI measured between the first and second AP. Data are shown as the mean ± SEM. Statistical significance was calculated with an unpaired student's t-test (***B, C***). *p < 0.05.

In contrast to the BLA-projecting neurons, fear conditioning did not alter the maximum *dV/dt* ($p = 0.88$; Figure 6.4*B,C*) or the AP half-width ($p = 0.071$; Figure 6.5*A,B*) of the mPFC-projecting neurons. Also the AP threshold ($p = 0.17$) and amplitude ($p = 0.30$; Table 6.2) as well as the first ISI ($p = 0.38$) did not differ in neurons from COND and CTX mice (Figure 6.5*C*). These results indicate that contextual fear conditioning resulted in neuronal population specific changes in the AP waveform, which were altered in the BLA- but not in the mPFC-projecting neurons. On the other hand fear conditioning did not alter the AP threshold or amplitude in either neuronal population.

6.5 Contextual fear conditioning increases the post-burst AHP in the BLA- but not in the mPFC-projecting neurons

The post-burst afterhyperpolarisation (AHP) is an important regulator of overall neuronal excitability (McKay et al, 2009). It is generated by the Ca^{2+} influx that occurs through voltage-gated Ca^{2+} channels during a train of APs. The rise in Ca^{2+} levels results in the activation of Ca^{2+} dependent K^+ currents, which then cause membrane hyperpolarisation (Abel et al., 2004; Stocker et al., 2004). Post-burst AHP consist of the medium (mAHP) and slow AHP (sAHP) components. The mAHP is activated within milliseconds following the AP and has a decay time in the range of 50-200 ms. The sAHP reaches its maximum peak over several hundred milliseconds and decays over the course of seconds (Sah & Faber, 2002; Stocker et al., 2004; Andrade et al., 2012). Learning dependent changes in the post-burst AHP have been shown to be induced by a variety of behavioural tasks including spatial learning and fear conditioning (Oh et al., 2003; McKay et al., 2009, Song et al., 2012; Sehgal et al., 2014; Disterhoft & Oh, 2006).

The amplitude of the mAHP is mediated by the number of AP fired in a train, with more APs resulting in increased amplitudes of the mAHP. However, for a fixed number of APs, the mAHP becomes larger at higher firing frequencies (Abel et al., 2004). Therefore, in order to investigate the effect of fear conditioning on the mAHP, we evoked 5 AP at frequencies ranging from 20-100 Hz. The mAHP was measured as the maximum negative peak following the last AP in the train. Figure 6.6*A* shows the representative voltage responses of BLA- and mPFC-projecting neurons from COND and CTX mice to 2 ms current pulses of 2 nA delivered at 100 Hz. We found that fear conditioning caused an increase in the mAHP amplitude in the BLA-projecting neurons at all frequencies between 60-100 Hz ($p <$

0.05; Figure 6.6*B*). Additionally, in agreement with previous research (Abel et al., 2004) we found a significant effect of AP frequency on the amplitude of mAHP ($p < 0.0001$).

In order to measure the sAHP we used a previously described protocol (McKay et al., 2009) in which a train of 15 APs was elicited at 50 Hz. The sAHP was measured 1 s after the end of the last AP (Figure 6.6*C*). Similarly to the mAHP, the sAHP was significantly increased in BLA-projecting neurons from COND mice when compared to those from CTX mice ($p = 0.02$; Figure 6.6*D*).

In contrast to the BLA-projecting neurons, the behavioural group had no effect on the amplitude of the mAHP in the mPFC-projecting neuronal population ($p = 0.91$; Figure 6.6*B*). However, as expected we found a significant effect of AP frequency on the amplitude of the mAHP ($p < 0.0001$). No differences in the amplitude of the sAHP were present between COND and CTX mice in the mPFC-projecting neurons ($p = 0.60$; Figure 6.6*D*). These results confirm that the AP firing frequency mediates the amplitude of the mAHP. More importantly, however, fear conditioning causes an increase in the amplitude of mAHP and sAHP, but does so only in the BLA-projecting neurons. The mPFC-projecting neurons are not affected by behavioural training.

Taken together, these results show that contextual fear conditioning causes neuronal-population specific changes in intrinsic excitability. This is marked by a depolarised shift in the RMP, a slower maximum *dV/dt,* longer AP half-width and an increased post-burst AHP. These changes are limited to the BLA-projecting neurons and are absent in mPFC-projecting neurons.

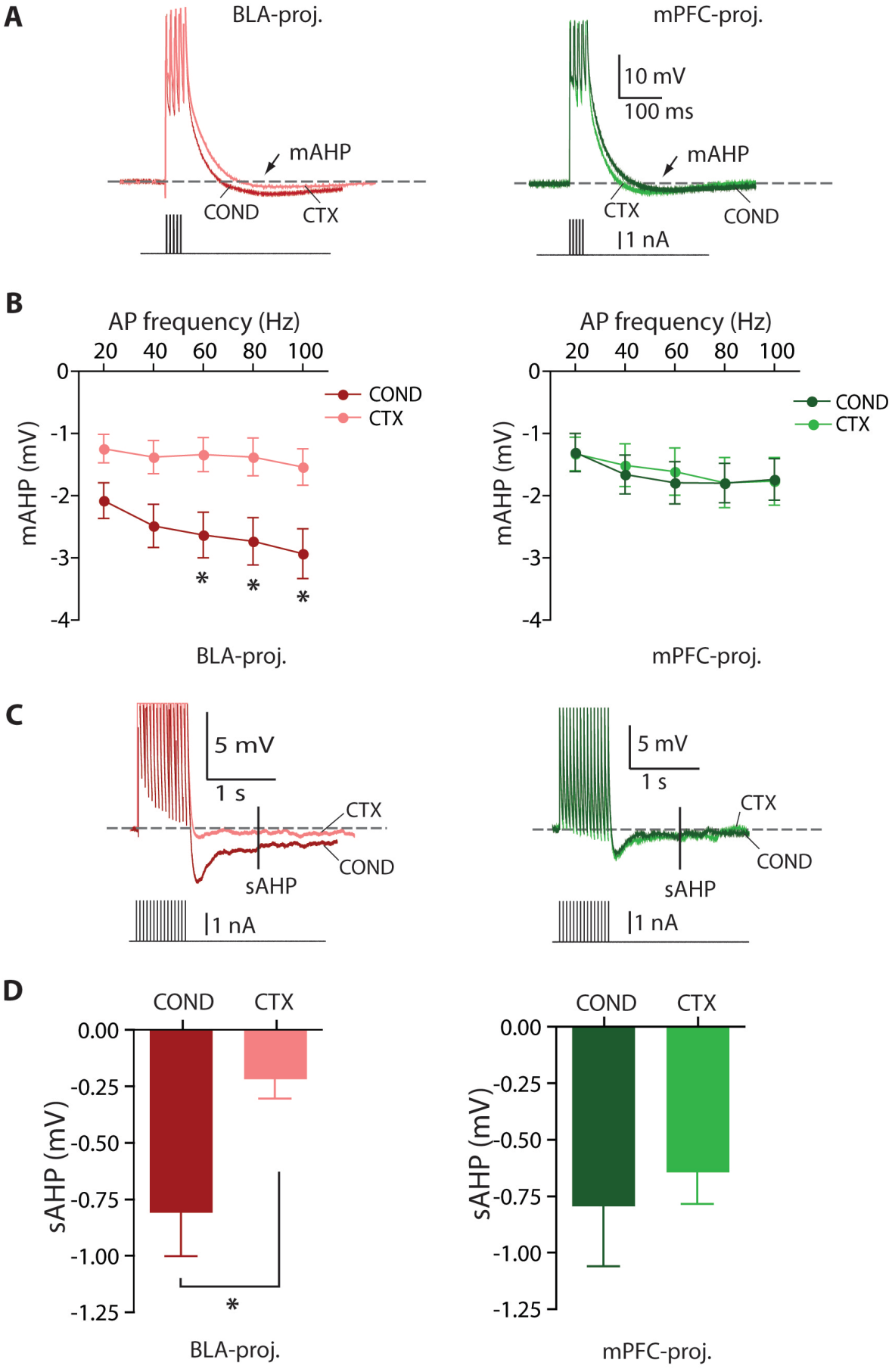
A
BLA-proj.
mAHP
COND
CTX
mPFC-proj.
10 mV
100 ms
mAHP
CTX
COND
1 nA
B
AP frequency (Hz)
20 40 60 80 100
0
-1
-2
-3
-4
mAHP (mV)
COND
CTX
*
*
*
BLA-proj.
AP frequency (Hz)
20 40 60 80 100
0
-1
-2
-3
-4
mAHP (mV)
COND
CTX
mPFC-proj.
C
5 mV
1 s
CTX
COND
sAHP
1 nA
5 mV
1 s
CTX
COND
sAHP
1 nA
D
COND
CTX
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
sAHP (mV)
*
BLA-proj.
COND
CTX
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
sAHP (mV)
mPFC-proj.

Figure 6.6. Fear conditioning induces a neuronal population specific increase in the post-burst AHP *A*, Example traces showing the voltage change in response to 2 ms current pulses of 2 nA at 100 Hz in BLA- (red) and mPFC-projecting (green) neurons from COND and CTX mice (traces are shown as an average of 3 repetitions, APs are truncated). The mAHP was measured as the maximum negative peak following the last AP in train (black arrow). ***B*,** Fear conditioning increased the amplitude of the mAHP in the BLA-projecting neurons ($F_{1,76} = 6.52$; $p = 0.0194$). This difference was most pronounced at frequencies between 60-100 Hz ($p < 0.05$). There was also a significant increase in the mAHP amplitude as a function of AP frequency ($F_{4,76} = 14.88$; $p < 0.0001$) as well as a significant frequency by group interaction ($F_{4,76} = 4.48$; $p < 0.0026$). In contrast, fear conditioning had no effect on the amplitude of the mAHP in the mPFC-projecting neurons ($F_{1,84} = 0.01$; $p = 0.91$). However, the mAHP amplitude was significantly affected by changes in AP firing frequency ($F_{4,84} = 13.95$; $p < 0.0001$) ***C*,** Example traces showing 15 APs at 50 Hz evoked by 2 ms current pulses of 2 nA, in the BLA- (red) and the mPFC-projecting (green) neurons from COND and CTX mice (APs are truncated). The sAHP was measured at 1 s following the last AP in train (black line). ***D*,** BLA-projecting neurons from COND mice (-0.81 ± 0.20 mV, $n = 11$) showed an increase in sAHP when compared to those from CTX mice (-0.22 ± 0.09 mV, $n = 9$, $t(18) = 2.55$, $p = 0.02$). This difference was not present when comparing neurons from COND (-0.79 ± 0.27, $n = 9$) and CTX mice (-0.64 ± 0.14, $n = 12$) in the mPFC-projecting group ($t(19) = 0.53$, $p = 0.60$). Data are shown as the mean ± SEM. Statistical significance was calculated by a two-way mixed-design ANOVA with Bonferroni's multiple comparisons test (***B*)** or an unpaired student's *t*-test (***D***). *$p < 0.05$.

6.6 Changes in the excitability of BLA-projecting neurons are learning-dependent

We found that contextual fear conditioning causes long-lasting changes in the intrinsic excitability of BLA-projecting PL neurons. However, the footshock that the mice are exposed to during training is also a stressful and mildly painful experience. It is, therefore, not clear whether the observed changes in excitability are learning-dependent or whether they arise from the pain or stress associated with the footshock. This is an important consideration given the fact that neurons in the mPFC are known to play a role in the experience of pain (Johansen et al., 2001). Moreover, some mPFC neurons have been shown to respond selectively to the expectation of aversive events (Baeg et al., 2001; Gilmartin & McEchron, 2005). It was therefore the second aim of this project to investigate whether the observed changes in intrinsic excitability were due to learning or whether they could have resulted from the aversive experience of the footshock. Because no differences in intrinsic excitability were found in the mPFC-projecting neuronal population, we limited our investigation to BLA-projecting neurons.

In order to control for the aversive effect of the footshock on neuronal excitability we introduced a second control group – the IMM group (Figure 6.1*A*). In contrast to COND mice, IMM mice do not form an association between the context and the aversive event (as measured by freezing) and this is known as the immediate shock deficit (Fanselow, 1986;

Landeira-Fernandez et al., 2006). Using this control allowed us to compare the changes in intrinsic plasticity of neurons from mice that had successfully undergone conditioning to those that did not despite having been exposed to the same aversive stimulus (Figure 6.1*B,C*).

The analyses of the subthreshold parameters of intrinsic excitability revealed that neurons from COND mice had significantly more depolarised RMP compared to those from IMM mice ($p < 0.05$). No statistically significant difference was present between the RMP of CTX and IMM mice ($p \geq 0.05$; Table 6.3). We found no significant between-group differences in the: R_N ($p = 0.54$), f_R ($p = 0.40$), sag ratio ($p = 0.76$), membrane τ in both the depolarising ($p = 0.063$) and hyperpolarising directions ($p = 0.79$) and in the rheobase ($p = 0.50$; Table 6.3). These results indicate that the depolarised shift in the RMP was specific to neurons from COND mice. On the other hand fear conditioning did not alter any of the other subthreshold parameters measured.

Table 6.3 Electrophysiological properties of BLA-projecting neurons, including the IMM group

	IMM (n = 11)[c]	COND (n = 14)[b]	CTX (n = 10)[b]
RMP (mV)	**-68 ± 1.29***	**-65 ± 0.71**[a]	-69 ± 0.83
R_N (mΩ)	175 ± 7.67	168 ± 11.36	158 ± 9.62
f_R (Hz)	0.56 ± 0.08	0.74 ± 0.13	0.53 ± 0.12
Sag ratio (%)	8.26 ± 1.03	8.99 ± 1.09	7.79 ± 1.42
Membrane τ depolarising (ms)	23.11 ± 0.69	26.03 ± 1.24	26.76 ± 1.05
Membrane τ hyperpolarising (ms)	25.30 ± 1.80	24.94 ± 1.12	26.46 ± 1.94
Rheobase (pA)	58 ± 4.23	67 ± 6.50	60 ± 5.97
AP threshold (mV)	-39 ± 0.89	-36 ± 0.48[a]	-38 ± 0.99
AP amplitude (mV)	83 ± 0.83	81 ± 1.25	83 ± 0.95
AP max *dV/dt* (mV/ms)	**351 ± 9.92***	**318 ± 9.06**	346 ± 5.89
AP half-width (ms)	**0.99 ± 0.02***	**1.10 ± 0.03**	1.00 ± 0.02

[a]For these parameters n = 12. Values for 2 cells were excluded due to offset problems.
[b]Values are the same as those presented in Table 6.1
[c]Statistical significance was calculated using a one-way ANOVA with Dunnett's multiple comparisons test where neurons from the IMM mice were compared to those from COND and CTX mice.
Data are shown as the mean ± SEM. *$p < 0.05$ (IMM compared to COND).

We next compared the number of APs fired by neurons from IMM, COND and CTX mice to depolarising current pulses of incrementing intensities and the maximum number of APs elicited by these pulses. Figure 6.7*A* shows some example responses of BLA-projecting neurons from COND and IMM mice to fixed-duration current step injections. We found no effect of contextual fear conditioning on the number of APs fired in response to depolarising steps at any current intensity ($p = 0.24$; Figure 6.7*B*). Similarly, the maximum number of APs elicited was comparable in neurons from all three behavioural groups ($p = 0.27$; Figure 6.7*C*).

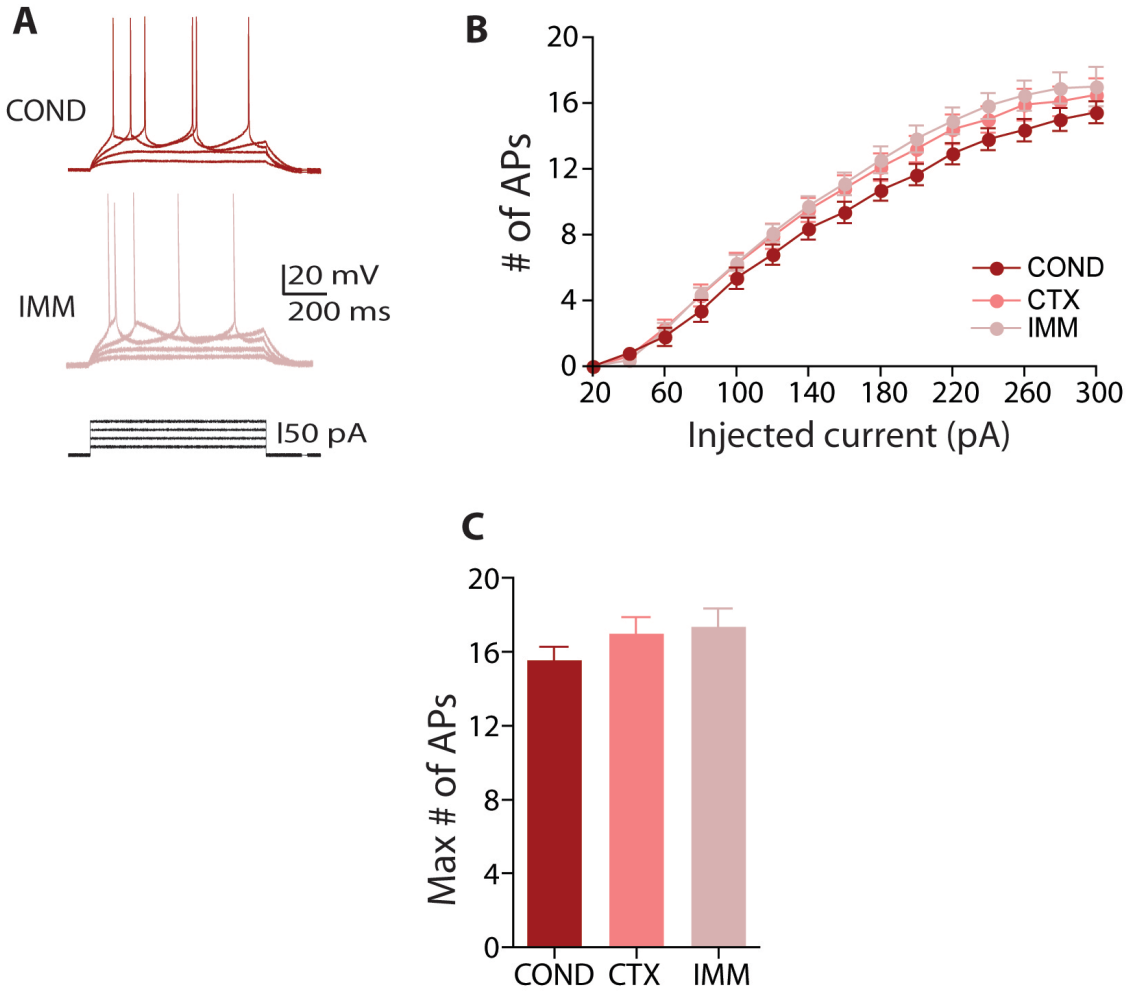

Figure 6.7 Contextual fear conditioning does not alter the number of APs fired by BLA-projecting neurons from all three behavioural groups. ***A,*** Example traces for BLA-projecting neurons from COND and IMM mice in response to 800 ms step current injections of 20 to 80 pA at 20 pA increments. ***B,*** There was no difference in the number of APs fired in response to depolarising steps of different current intensities between neurons from COND, CTX and IMM mice ($F_{2,448} = 1.50$, $p = 0.24$). ***C,*** The maximum number of APs elicited did not differ between neurons from COND (16 ± 0.72, $n = 14$), CTX (17 ± 0.87, $n = 10$) and IMM mice (17 ± 0.99, $n = 11$; $F_{2,32} = 1.35$, $p = 0.27$). Data are shown as the mean ± SEM. Statistical significance was calculated using a two-way mixed-design ANOVA (***B***) or a one-way between-subjects ANOVA (***C***). Data for COND and CTX mice are the same as those presented in **Figure 6.3**

We then moved on to analysing the AP kinetics. Figure 6.8*A* shows representative traces of the first AP in BLA-projecting neurons from COND, CTX and IMM mice with sufficient current to trigger four APs in 800 ms with the corresponding phase plots presented in Figure 6.8*B*. We found that neurons from the COND mice had a significantly slower maximum *dV/dt* compared to those from the IMM mice ($p < 0.05$). There were no differences between the maximum *dV/dt* of neurons from CTX and IMM mice ($p \geq 0.05$; Figure 6.*8B,C*). Additionally we found that neurons from the COND group had significantly longer AP half-widths compared to those from the IMM group ($p < 0.05$). There was no significant difference in the AP half-width between the neurons from CTX and IMM mice ($p \geq 0.05$; Figure 6.8*D*,*E*). Fear conditioning did not alter the AP threshold ($p = 0.068$) or amplitude ($p = 0.51$; Table 6.3). Finally, the analysis of the first ISI did not reveal any differences between neurons from IMM, COND and CTX mice although a strong trend towards significant inter-group differences was present ($p = 0.0522$; Figure 6.8*F*). These results show that changes in the AP kinetics occur only in neurons from COND mice and are absent in neurons from CTX and IMM mice. Fear conditioning, however has no effect on the AP threshold or amplitude.

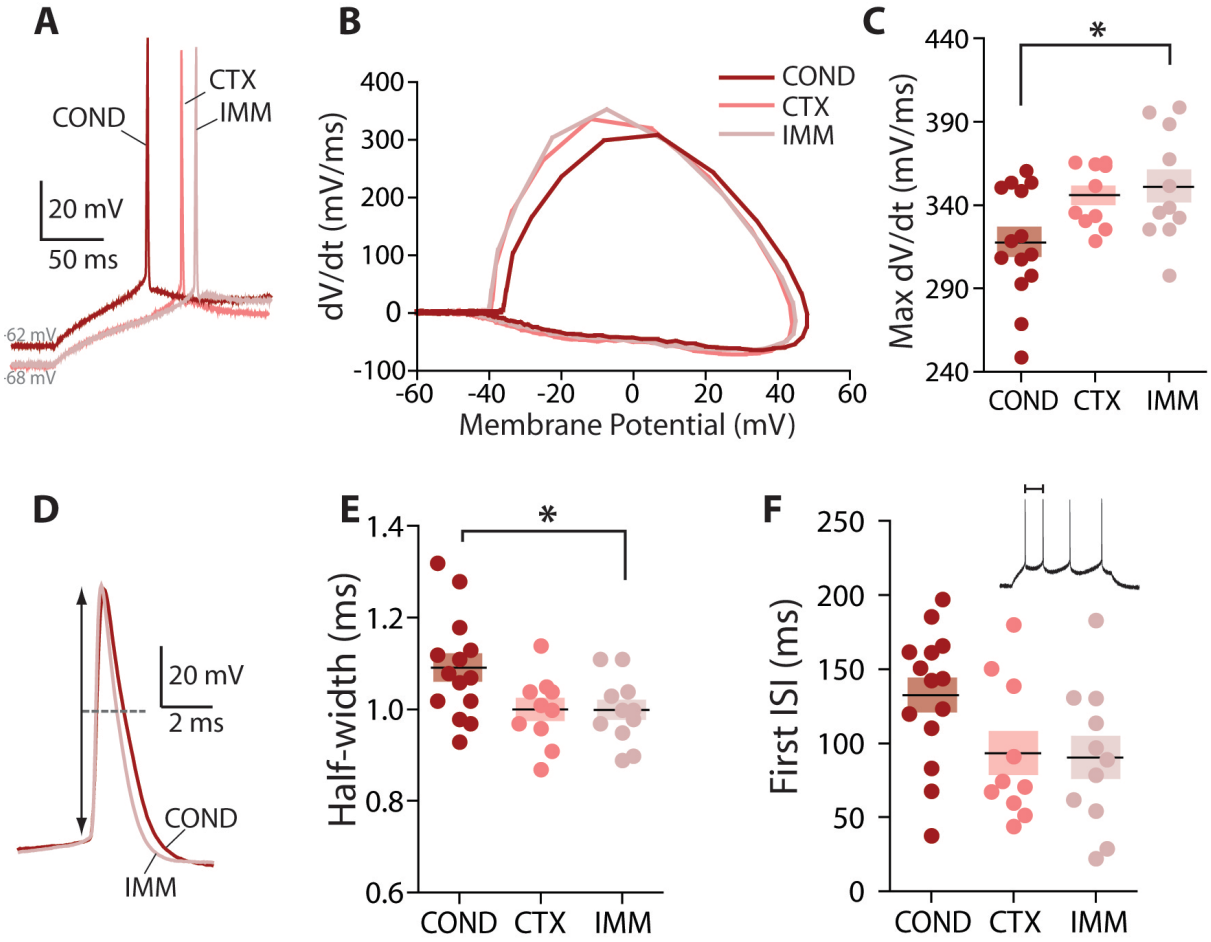

Figure 6.8 Fear conditioning induced changes in AP kinetics are learning-dependent. ***A,*** Example traces showing the first AP fired by BLA-projecting neurons from both COND and IMM mice in response to a 800 ms step current injection sufficient to evoke 4 APs. ***B,*** Phase plot for the corresponding APs shown in (***A***). The maximum *dV/dt* was measured as the peak of the phase plot. ***C,*** The maximum *dV/dt* was reduced in neurons from COND mice (318 ± 9.06 mV/ms, $n = 14$) when compared to those from IMM mice (351 ± 9.91 mV/ms, $n = 11$; $p < 0.05$). There was no difference in the maximum *dV/dt* between neurons from CTX (346 ± 5.89 mV/ms, $n = 10$) and IMM mice ($p \geq 0.05$; $F_{2,32} = 4.54$, $p = 0.02$) ***D,*** Example traces showing the AP half-width measured at half the distance between the AP threshold and peak for BLA-projecting neurons from both COND and IMM mice. ***E,*** The AP half-width differed significantly between COND (1.09 ± 0.03 ms, $n = 14$), CTX (1.00 ± 0.02 ms, $n = 10$) and IMM mice (1.00 ± 0.02; $n = 14$) following fear conditioning ($F_{2,32} = 4.25$, $p = 0.02$). Neurons from COND mice had significantly increased AP half-widths when compared to those from IMM mice ($p < 0.05$). No difference was present in the AP half-width of neurons from CTX and IMM mice ($p \geq 0.05$). ***F,*** Fear conditioning did not alter the first ISI of neurons from COND (133 ± 12 ms, $n = 14$), CTX (93 ± 15 ms, $n = 10$) and IMM mice (90 ± 15 ms, $n = 11$) although a strong trend towards significantly different group means was present ($F_{2,32} = 3.24$, $p = 0.0522$). The inset is an example trace showing the ISI measured between the first and second AP. Data are shown as the mean ± SEM. Statistical significance was calculated using a one-way between-subjects ANOVA with Dunnett's multiple comparisons test (***C,E,F,*** IMM compared to COND and CTX), *$p < 0.05$. Data for COND and CONT mice are the same as those presented in **Figure 6.4 and Figure 6.5**

Finally, we analysed the amplitude of the mAHP and sAHP. Figure 6.9*A* shows the representative voltage responses of BLA-projecting neurons from COND and IMM mice to 2 ms current pulses of 2 nA delivered at 100 Hz. We found that cells from the COND mice had increased mAHP when compared to those from CTX mice at all frequencies between 40-100 Hz (40-60 Hz: $p < 0.05$, 80-100 Hz: $p < 0.01$). No significant differences in the mAHP were present between neurons from CTX and IMM mice (Figure 6.*9B*). Moreover, as before we found a significant effect of the AP frequency on the amplitude of the mAHP ($p < 0.0001$). Similarly to the mAHP, the sAHP was significantly larger in neurons from COND mice when compared to those from IMM mice ($p < 0.05$). There was no significant difference between the sAHP of neurons from CTX and IMM mice ($p \geq 0.05$; Figure 6.9*C,D*). These results indicate that the increase in the post-bust AHP observed in the BLA-projecting neurons following fear conditioning was learning-dependent.

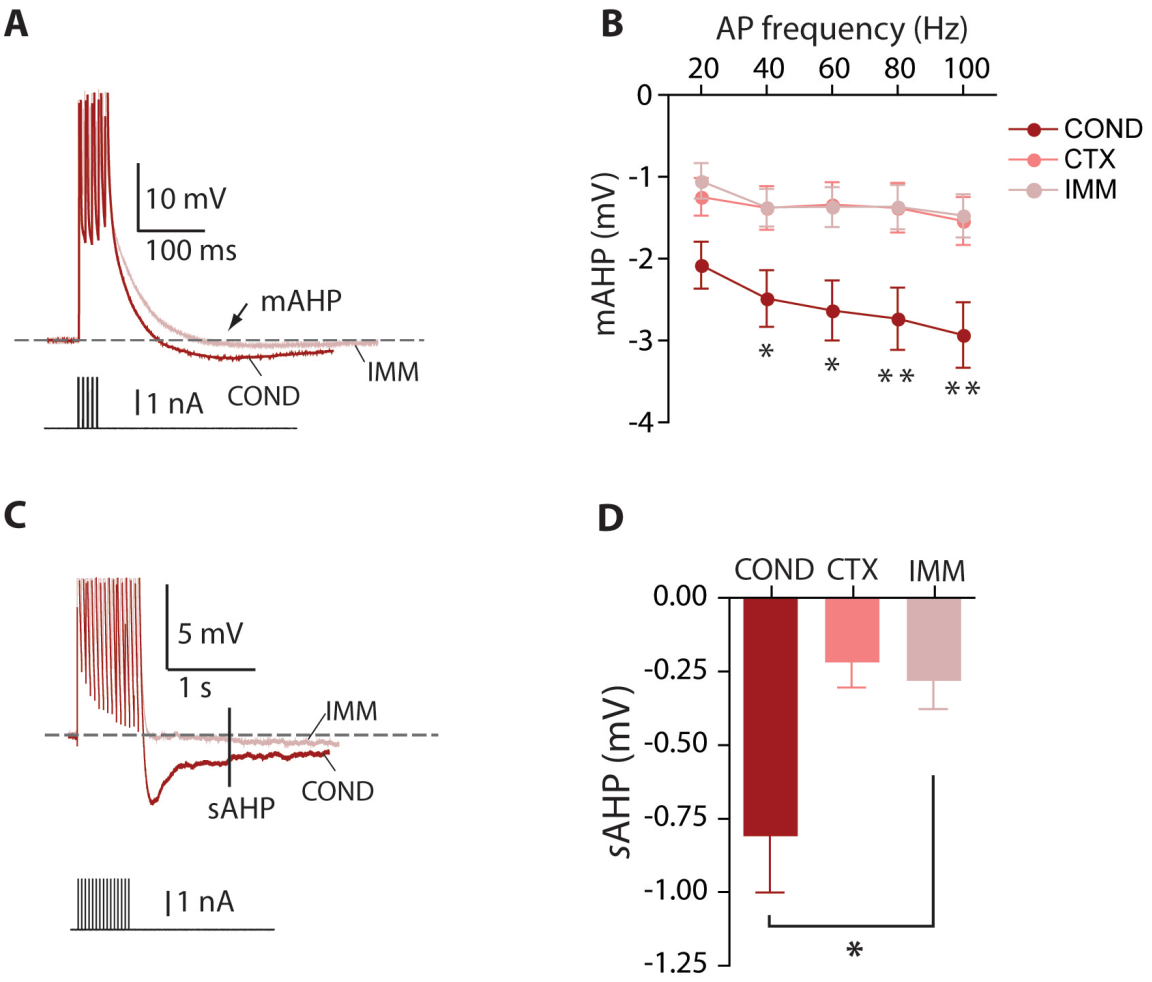

Figure 6.9. Fear conditioning induces a learning-dependent increase in the post-burst AHP. ***A,*** Example traces showing a train of APs fired at a 100 Hz by BLA-projecting neurons from COND and IMM mice in response to 2 ms current pulses of 2 nA (traces are shown as an average of 3 repetitions, APs are truncated). The mAHP was measured as the maximum negative peak following the last AP in train (black arrow). ***B,*** Mean amplitude of the mAHP across 20-100 AP frequencies for all behavioural groups. The amplitude of the mAHP was significantly larger in neurons from COND mice when compared to those from IMM mice at AP frequencies between 40-100 Hz (40-60 Hz: $p < 0.05$, 80-100 Hz: $p < 0.01$). No significant differences in the amplitude of the mAHP were present between neurons from CTX and IMM mice ($p \geq 0.05$; $F_{2,116} = 5.85$; $p = 0.0073$). There was a significant main effect of the AP frequency on the mAHP amplitude ($F_{4,116} = 23.41$; $p < 0.0001$) and a significant frequency by group interaction ($F_{8,116} = 3.34$; $p = 0.0018$). ***C,*** Example traces showing 15 APs at 50 Hz elicited by 2 ms current pulses of 2 nA in the BLA-projecting neurons from COND and IMM mice (APs are truncated). The sAHP was measured at 1s following the last AP in train (black line). ***D,*** The sAHP in neurons from COND mice (-0.81 ± 0.20 mV, $n = 11$) was larger when compared to that from IMM mice (-0.28 ± 0.10 mV, $p < 0.05$). No difference in the sAHP amplitude was present in neurons from CTX (-0.22 ± 0.09 mV, $n = 9$) and IMM mice ($p \geq 0.05$; $F_{2,28} = 5.32$, $p = 0.011$). Data are shown as the mean ± SEM. Statistical significance was calculated using a two-way mixed-design ANOVA with Bonferroni's multiple comparisons tests (***B***) or a one-way between-subjects ANOVA with Dunnett's multiple comparisons test (IMM compared to COND and CTX; ***D***), *$p < 0.05$, **$p < 0.01$ (IMM compared to COND). Data for COND and CTX mice are the same as those presented in **Figure 6.6.**

To summarise, we have found that fear conditioning results in learning-dependent changes in the intrinsic properties of PL neurons. These are: a depolarised shift in the RMP, a slower maximum *dV/dt*, a longer AP half-width and an increase in the post-burst AHP. We know that these changes are learning dependent because they occur only in neurons from COND mice and are absent in those from CTX and IMM mice. Furthermore, we have demonstrated that changes in intrinsic excitability occur in the BLA-projecting but not in the mPFC-projecting neurons. This indicates that learning-induced intrinsic plasticity does not occur globally in the PL but is instead limited to specific neuronal populations, which can be distinguished based on the neurons' long-range projection targets.

7. Discussion

The first aim of this project was to explore whether learning alters the intrinsic excitability of the prelimbic neurons (PL) that project to the ipsilateral basolateral amygdala (BLA) or to the contralateral medial prefrontal cortex (mPFC). For this purpose we combined contextual fear conditioning with retrograde labelling and patch-clamp electrophysiology. We demonstrated that: (1) contextual fear conditioning does alter the intrinsic excitability of PL neurons, (2) these changes are neuronal population specific - plasticity occurred in the BLA-projecting neurons but was absent in the mPFC-projecting neurons. The second aim of the project was therefore to determine whether the observed changes in intrinsic properties were learning-dependent or whether they could have instead been induced by stress and/or pain associated with the footshock. In order to address this, we introduced a second behavioural control group in which the mice were exposed to the same number of footshocks as the conditioned group (COND) but instead failed to form an aversive memory of the context. By adding the immediate shock group (IMM) we showed that the observed changes in intrinsic properties were indeed learning-dependent – they occurred only in neurons from COND mice and were absent in neurons from both context only (CTX) and IMM mice. These findings therefore indicate that learning in a contextual fear conditioning task induces intrinsic plasticity of PL neurons that is not generalised across all neurons but is instead limited to specific neuronal populations.

7.1 Fear conditioning induces intrinsic plasticity in the BLA- but not in the mPFC-projecting PL neurons

Fear conditioning has been shown to induce intrinsic plasticity in a number of brain regions including the hippocampus (McKay et al., 2009; Song et al., 2012), the BLA (Sehgal et al., 2014; Senn et al., 2014), and the infralimbic cortex (IL; Santini et al., 2008). Our results confirm that fear conditioning alters neuronal intrinsic excitability and we expand on the above findings by showing that these changes also occur in the PL. More importantly, by investigating two distinct neuronal populations we show that the BLA-projecting but not the mPFC-projecting neurons are altered by fear conditioning. This finding is in agreement with other recent research showing that fear conditioning preferentially recruits selected neuronal

populations within the same brain region based on the neurons' long-range projection targets (Knapska et al., 2012; Senn et al., 2014).

The mPFC is an important region for the acquisition and storage of long-term fear memories (Frankand et al., 2004; Einarsson and Nader, 2012) as well as the executive control over emotional responses (Fuster 2008; Euston et al., 2012). While individual mPFC subregions contribute differently to the processing of fear memories and extinction, the PL seems to be of particular importance for mediating the expression of learned fear (Vidal-Gonzales et al., 2006; Corcoran and Quirk, 2007; Laurent and Westbrook, 2009; Sierra-Mercado et al., 2011; Burgos-Robles et al., 2009; Fenton et al., 2014). Consequently, any change in the intrinsic properties of PL neurons could alter the manner in which fear responses are generated. This is particularly relevant in the context of the BLA-projecting neurons. The PL is bi-directionally connected to the BLA (McDonald, 1991, 1996; Vertes, 2004) and this pathway is important for fear learning and expression (Corcoran and Quirk, 2007; Stevenson, 2011; Vouimba and Maroun, 2011). Indeed, fear conditioning has been shown to increase the amplitude of evoked field potentials at the PL-BLA pathway and this increase correlates positively with freezing levels during memory retrieval (Vouimba and Maroun, 2011). Furthermore, the disruption of the communication between the PL and the BLA results in impairments in the expression of fear memory (Steveneson, 2011). Finally, the BLA-projecting PL neurons have been shown to be preferentially recruited during states of high fear (Orsini et al., 2011). Given the above evidence, any alteration in the excitability of the BLA-projecting PL neurons could therefore influence BLA-mediated fear expression and/or memory allocation to these neurons.

In spite of seeing a learning-induced intrinsic plasticity in the BLA-projecting neurons we found no effect of fear conditioning on the intrinsic properties of the mPFC-projecting neurons. One possibility for this finding could simply be that these neurons are not recruited by fear conditioning. Perhaps interhemispheric communication between mPFC neurons is more critical in tasks requiring higher cognitive processing such as working memory, error detection or decision making (Euston et al., 2012). Another possibility is that these neurons do play an important role in fear memory but do so at more remote time points. Indeed, the mPFC is important for the consolidation, storage and retrieval of remote long-term memories. For example, spine growth in the mPFC has been shown to be necessary for the consolidation of contextual fear memories and this increase does not become evident until approximately one week after conditioning (Restivo et al., 2009; Vetere et al., 2011). Furthermore, remote,

but not recent, memory retrieval results in increased metabolic activity (Bontempi et al., 1999) and immediate early gene expression in the mPFC (Frankland et al., 2004; Maviel et al., 2004). Finally, the lesioning or the inactivation of the mPFC blocks remote memory retrieval in a variety of behavioural paradigms (Takehara et al., 2003; Frankland et al., 2004; Maviel et al., 2004; Teixeira et al., 2006; Ding et al., 2008; Oswald et al., 2010; Lesburguères et al., 2011). A scenario in which the mPFC-projecting neurons are recruited at more remote time points seems therefore plausible. On the other hand, the early involvement of the BLA-projecting neurons makes perfect sense. Fear memory is available for recall as early as 2 h following conditioning (Burgos-Robles et al., 2009) suggesting that the involvement of the PL-BLA pathway is necessary from very early time points.

7.2 Fear conditioning causes a depolarised shift in the resting membrane potential

Our results show that BLA-projecting neurons display more depolarised resting membrane potentials (RMP) following fear conditioning, while no changes are present in other subthreshold membrane parameters (Table 6.1 and 6.3, Chapter 6). This change in RMP is in agreement with other studies that have found a positive shift in the RMP following associative learning (Gainutdinov et al., 1998; Kemenes et al., 2006).

The RMP is an important mediator of the overall neuronal excitability. For example, more depolarised RMP can enhance cell excitability by bringing the membrane potential closer to the action potential (AP) firing threshold, which results in an increased AP discharge to a given current injection. Moreover, because voltage-gated ion channels depend on the membrane voltage, a shift in the RMP can produce changes in the neuronal input resistance and enhance neuronal firing independently of the AP threshold (Dougherty et al., 2012). However, in our study the depolarised shift in the RMP was not accompanied by changes in other subthreshold membrane parameters or by an increase in neuronal firing (Table 6.1 and 6.3; Figure 6.3 and 6.7; Chapter 6). Interestingly, similar results have been reported in a study on the cerebral giant cells of a snail, where a single-trial associative learning paradigm resulted in a depolarised shift in the RMP that emerged between 16-24 h following conditioning and persisted for at least 14 days. At baseline conditions, this depolarisation was not accompanied by changes in neuronal input resistance or firing frequency. However, upon exposure to the conditioned stimulus (CS) the depolarised RMP caused an increase in neuronal firing, showing that a change in the RMP was sufficient to drive an increase in the

network's response to the CS (Kemenes et al., 2006). In the context of our paradigm, it could therefore be hypothesised that the depolarised RMP could contribute to/drive increased firing upon re-exposure to the conditioning environment.

Another possible function for a shift in the RMP is to regulate subsequent neuronal excitability and synaptic plasticity. For example, prolonged AP firing of lateral amygdala (LA) neurons can rapidly decrease membrane excitability *in vivo* by reducing the number of APs evoked by subsequent depolarising current steps. Interestingly, this reduction in excitability is sensitive to shifts in the membrane potential. When LA neurons are stimulated at more hyperpolarised RMPs to induce bursting in the theta rhythm, the observed decrease in excitability is only transient. However, when the membrane potential is depolarised to more physiological levels, the same stimulation results in a robust decrease of excitability. This decrease in intrinsic excitability reduces the efficacy of subsequently induced synaptic LTP (Rosenkranz, 2011). Given the fact that changes in intrinsic excitability can readily control the induction of synaptic plasticity *in vivo* what role could this serve in the intact brain? A possible physiological function for this phenomenon is to reduce subsequent associative learning while leaving previous plasticity intact. This would have important implications for the formation of long-term memories and could act as a mechanism that is used during heightened periods of activity (Rosenkranz, 2011), such as fear conditioning.

7.3 Fear conditioning causes changes in the AP waveform

In addition to a more depolarised RMP, we found a change in the AP waveform. We observed that fear conditioning reduced the maximum velocity of the upstroke phase of the AP. This was manifested by a slower maximum rate of depolarisation (*dV/dt*) in neurons from COND mice when compared to those from CTX and IMM (Figure 6.4 and 6.8, Chapter 6).

The rising phase of the AP occurs due to the 'explosive' and regenerative activation of the Na^+ inward current resulting in rapid membrane depolarisation. Measurements taken at the maximum *dV/dt* can be a direct estimation of the maximum Na^+ current flowing during the AP upstroke phase, when contributions from other channels are likely to be small (Bean, 2007). A reduction in the value of the maximum *dV/dt* indicates a decrease in Na^+ channel availability either through a decrease in the channel density or a change in the channel properties. Indeed, it has been shown that repeated neuronal depolarisation can result in an activity-dependent decrease in the maximum *dV/dt* that is mediated by a reduction in Na^+

currents. This effect is most pronounced in the dendrites but can nonetheless be observed in the soma. Importantly, the decrease in Na^+ currents is greater at more depolarised membrane potentials (Colbert et al., 1997), suggesting that the depolarised shift in the RMP that we observed could at least partially contribute to the reduction of the maximum *dV/dt*.

Apart from a decrease in the maximum *dV/dt* we also found a significant broadening of the AP half-width in COND mice when compared to CTX and IMM mice (Figure 6.5 and 6.8, Chapter 6). These results are in agreement with other studies that have also shown a learning-induced increase in the AP width (Matthews et al., 2008; Senn et al., 2014).

Neuronal output has been typically perceived as a binary code with graded synaptic potentials being converted into all or nothing AP. However, it is now becoming clear that the AP waveform could be an important carrier of information and is subject to plasticity changes (Debanne, 2004). Indeed, the same pyramidal neurons are capable of producing different AP shapes depending on previous stimulus history and conductances, with broader AP encoding larger stimulus amplitudes. This 'AP waveform code' was found to be highly reliable, resulting in increased information transfer when compared to AP timing alone (Polavieja et al., 2005; Juusola et al., 2007).

Action potential repolarisation and hence duration is controlled by several classes of K^+ channels. In the cell body these are: the voltage-gated Kv4 channels, which mediate the A-type current, Kv3 channels and the large conductance BK channels, which require both intracellular Ca^{2+} and membrane depolarisation for their activation (Sah & Faber, 2002; Kim et al., 2005; Bean, 2007). In the axon, the width of the AP is controlled largely by voltage-gated Kv1 and Kv3 channels (Geiger and Jonas, 2000; Kole et al., 2007; Debanne et al., 2011). The broadening of the AP is probably most important at the level of axon terminals, where even small differences in the AP width can produce significant changes in the timing and strength of synaptic transmission (Bean, 2007). For example Geiger & Jonas (2000) showed that APs in the mossy fibre terminal undergo activity-dependent broadening as a consequence of repetitive theta burst stimulation. This is due to a decreased rate of repolarisation mediated by the inactivation of low-threshold voltage-gated Kv1 channels. On the functional level, the broadening of the presynaptic AP enhances Ca^{2+} influx through voltage-gated Ca^{2+} channels resulting in an increased neurotransmitter release and an increase in the amplitude of excitatory postsynaptic currents (EPSP, Geiger & Jonas, 2000).

At the somatic level a decrease in BK channel activity can result in a significant broadening of the AP and in turn lead to elevated presynaptic Ca^{2+} influx and an increase in

synaptic transmission in nearby neurons (Deng et al., 2013). Importantly, learning-dependent increases in intrinsic excitability can in part be mediated by a decrease in BK currents (Matthews et al., 2008). The fact that the kinetics of single APs and the associated synaptic transmission are sensitive to recent activity and the general network state, suggests that neurons might convey messages through a mix of both analogue and digital signals (Alle and Geiger, 2006; Kole et al., 2007; Shu et al., 2007).

The exact shape of the AP can differ between the soma and the axon (Geiger & Jonas, 2000; Kole et al., 2007). For example, Kole et al., (2007) demonstrated that in neocortical layer 5 neurons the width of the AP recorded at the soma decreases steeply in the first 50 micrometres of the axon initial segment (AIS) after which it remains constant as it spreads throughout the axonal arbour. This dynamic reduction in width is due to the slowly inactivating Kv1 channels present in high density in the AIS. The somatic and axonal AP waveform are also modulated in a highly compartmentalised and independent fashion, with rapid high-frequency AP bursts causing the broadening of somatic AP and sustained subthreshold activity selectively increasing the axonal AP duration (Kole at al., 2007). However, under certain conditions, such as subthreshold somatic depolarisations, the somatic AP waveform can survive the initial axonal filtering and propagate over much longer distances along the axon. Indeed, when steady-state somatic depolarisations precede the AP, the duration of the AP increases rapidly in the soma and can propagate up to 400-600 microns into the axon. The resulting changes in the axonal APs occur more slowly than those observed in the soma but in agreement with previous observations also produce an increase in EPSPs in nearby neurons (Shu et al., 2006; Kole et al., 2007). The enhanced propagation of the somatic AP waveform is likely due to the passively spreading somatic depolarisation leading to the inactivation of axonal Kv1 channels (Kole et al., 2007; Shu et al., 2007). The effect of the axonal AP broadening is most pronounced at membrane potentials near the firing threshold but it is tempting to think that even a modest depolarisation could impact the shape of the axonal AP. This is important in the context of our study given the fact that neurons from COND mice have significantly more depolarised RMP when compared to those from CTX and IMM mice (Table 6.1 and 6.3). However, even if the more depolarised RMP does allow the AP shape to propagate further along the axon, it is unlikely to increase synaptic transmission at the PL-BLA synapse. Somatic depolarisation has been shown to influence the AP waveform up to approximately 400-600 microns along the axon, whereas the distance between the PL and BLA is in the order of several millimetres. However, even if the shape of

the somatically recorded AP does not travel all the way to the BLA, it could still have important functional consequences locally. Indeed, even modest subthreshold depolarisations can affect the shape of the AP in axon collaterals and *en passant* terminals of layer 5 cortical neurons (Foust et al., 2011), and cause an increase in the amplitude of EPSPs in nearby pyramidal cells (Alle & Geiger, 2006; Shu et al., 2006).

Apart from travelling forward along the axon, in certain neuronal types such as the hippocampal CA1 and cortical pyramidal neurons, APs also actively backpropagate into the dendrites (Stuart and Sakmann, 1994; Johnston et al., 1996; Stuart et al.,1997a, 1997b). Activity-dependent inactivation of somatic and dendritic Kv4.2 channels results in AP broadening and enhanced AP backpropagation. This in turn can impact the time course and degree of Ca^{2+} influx as well as the associated second messenger cascades, gene expression and synaptic plasticity (Frick et al., 2004; Kim et al., 2005; Chen et al., 2006). Additionally, the increase in AP width can also help shape future synaptic inputs. For example Häusser et al., (2001) found that backpropagating APs can serve as a negative feedback mechanism by shunting dendritic EPSPs and thus controlling the probability of the next AP generation. By examining two types of neurons - neocortical layer 5 neurons and Purkinje neurons - that have distinctly different AP waveforms, they found that the AP waveform affected the degree of EPSPs shunting with broader APs producing more shunting (Häusser et al., 2001). Moreover, a later study by Zhou et al. (2005) showed that the AP half-width measured at the soma correlates with the direction of synaptic modifications induced through a spike timing dependent plasticity protocol (STDP). STDP can be defined as a phenomenon in which the induction of either LTP or LTD depends on the precise temporal order of repeated pre- and postsynaptic APs (STDP; Dan and Poo, 2006). Zhou et al. (2005) found that experimentally induced broadening of the APs to over 1.5 ms resulted in a shift in the balance of STDP towards LTD. In our experiments the width of APs in COND animals rarely lasts longer than 1.1 ms and does not exceed 1.3 ms (Table 6.3; Figure 6.5 and 6.8, Chapter 6) so the results observed by Zhou et al. (2005) cannot be applied to our study. However the interaction between AP width and dendritic EPSPs is still worth noting.

Finally, the AP waveform can also be altered by rapidly induced structural plasticity of the AIS. Prolonged depolarisation of hippocampal cultured neurons, through incubation in a high KCl containing medium, results in the shortening of the AIS. This structural plasticity results in a slower maximum *dV/dt,* broader AP width, an increase in the first inter-spike-interval and a decrease in the number of APs fired. These changes are due to the structural

plasticity and not a change of somatic Na^+/K^+ channels (Evans et al., 2013). The activity-dependent changes in the AIS are most likely triggered by the activation of L-type Ca^{2+} channels (Grubb & Burrone, 2010; Evans et al., 2013).

7.4 Fear conditioning increases the post-burst afterhyperpolarisation

Our results showed that contextual fear conditioning increases the afterhyperpolarisation (AHP) occurring after a train of APs. This was manifested by an increase in both the medium (mAHP) and slow (sAHP) component (Figure 6.6 and 6.9, Chapter 6). Unexpectedly, our results do not support previous findings, most of which consistently show a decrease of post-burst AHP following learning in a variety of behavioural paradigms including trace-eyelid conditioning (Moyer et al., 1996; Thompson et al., 1996; Kuo et al., 2008; Oh et al., 2009), spatial learning in the Morris water maze (Oh et al., 2003), operant conditioning (Saar et al., 1998) as well as fear conditioning (McKay et al., 2009; Song et al., 2012; Sehgal et al., 2014). In fact, the post-burst AHP is considered to be a marker for successful learning and a lack of decrease has been associated with a failure to acquire the task (Moyer et al., 1996; Oh et al., 2003; Song et al., 2012).

Both mAHP and sAHP are generated by Ca^{2+} dependent K^+ currents that are activated by a Ca^{2+} influx that occurs during a train of APs (Sah & Faber, 2002; Abel et al., 2004). The pharmacological block of the small conductance Ca^{2+} activated K^+ channels (SK) facilitates learning (Messier at al., 1991; Deschaux et al., 1997; van der Staay et al., 1999; Stackman et al., 2002; Criado-Marrero et al., 2014). Moreover, the pharmacological activation or the genetic overexpression of SK2 channels - the specific channels underlying mAHP - reduce spontaneous firing rates *in vivo* and impair memory acquisition over a range of behavioural tasks, including contextual fear conditioning (Hammond et al., 2006; McKay et al., 2012; Stackman, Jr. et al., 2008; Vick et al., 2010). In slices, the activation or overexpression of SK2 channels increases the mAHP, decreases the number of APs as well as the synapatically evoked glutamatergic EPSPs, and attenuates LTP (Hammond et al., 2006; McKay et al., 2012; Criado-Marrero et al., 2014).

The exact effect of sAHP modulation on behaviour has been investigated to a lesser degree because the specific Ca^{2+} activated K^+ channels generating the current responsible for sAHP have yet to be identified (Sah & Faber, 2002; Stocker et al., 2004). It is known however, that the sAHP plays a role in the late phase spike frequency adaptation leading to a

strong reduction in AP firing (Madison & Nicoll, 1982; Stocker et al., 2004) and it might impair NMDA receptor mediated responses (Disterhoft et al., 2004). The channels mediating sAHP are thought to be important for memory encoding and a baseline increase in the sAHP in ageing animals is believed to underpin learning deficits (Disterhoft et al., 2004).

If the reduction in post-burst AHP is associated with successful learning why did we find an increase in both AHP components in COND mice? One possibility is that these mice did not successfully learn the context-shock association. However, this is unlikely for two reasons. Firstly, the percentage of successful learners in our fear conditioning paradigm is very high and constitutes approximately 70% (Figure 6.1, Chapter 6). Moreover, even though we cannot in all certainty exclude the possibility that the mice used for our recordings failed to learn the task, we regularly validated the behavioural protocol by testing memory recall in a subset of mice not used for electrophysiology. Secondly, in all the studies reported above, unsuccessful learning resulted in no change of the post-burst AHP relative to control animals. It could therefore be implied that non-learners should display similar intrinsic properties to control/naïve animals (Moyer et al., 1996; Oh et al., 2003; Song et al., 2012). This is clearly not the case in our experiments as the post-burst AHP is specifically increased in neurons from COND mice when compared to those CTX and IMM mice showing that the observed increase in post-burst AHP is not due to a failure to learn the context-shock association.

If the increase in post-burst AHP is learning-specific, what could be its physiological role? First of all, it is worth noting that the AHP plays an active role in regulating firing frequency – a reduction in AHP increases neuronal firing, whereas an increase in AHP causes a marked decrease in firing (Moyer et al., 1996; Thompson et al., 1996; Kuo et al., 2008; McKay et al., 2009; Song et al., 2012; Sehgal et al., 2014; McKay et al., 2012; Criado-Marrero et al., 2014). Surprisingly, in our study we find no difference in number of AP fired in response to depolarising current pulses (Figure 6.3 and 6.7, Chapter 6). However, given the fact that the effect of the post-burst AHP on regulating neuronal firing is well established (Madison & Nicoll, 1982; Sah & Faber, 2002; Stocker et al., 2004), it seems likely that in our study, the AHP could still have reduced neuronal firing. Instead of causing a marked reduction in the number of AP it might have alternatively acted as a feedback mechanism to compensate for neuronal overexcitability by bringing the firing rates down to control levels. Indeed, Ca^{2+} entry during repetitive firing provides the cell with a simple and precise indicator of its recent activity (Helmchen et al., 1996) and can act as a negative feedback system by activating AHP conductances (Wang, 1998). Additionally, apart from mediating post-bust

AHP, SK channels also play an important role in regulating synaptic transmission and plasticity in the prefrontal cortex (Faber & Sah, 2007). During basal synaptic transmission they are activated by Ca^{2+} influx through NMDA receptors as well as voltage-gated Ca^{2+} channels. Their activation results in the attenuation of excitatory synaptic transmission (Faber, 2010). The activation of SK channels could therefore act as a filtering mechanism to reduce unnecessary or conflicting synaptic input.

Another reason for the activation of the Ca^{2+}-depenent K^+ channels in our study could be linked to the specific role played by the PL in fear conditioning. Given the well established role of the PL in mediating the expression of conditioned fear (Vidal-Gonzales et al., 2006; Corcoran and Quirk, 2007; Laurent and Westbrook, 2009; Sierra-Mercado et al., 2011; Fenton et al., 2014), the activation of Ca^{2+}-depenent K^+ channels could act as a balancing force to prevent fear expression outside of the conditioning context. This mechanism would be of particular importance in the PL-BLA pathway, which mediates fear learning and expression (Stevenson, 2011; Vouimba and Maroun, 2011; Orsini et al., 2011).

7.5 Could the behavioural protocol and the neuronal population matter?

Many studies have investigated the effect of behavioural training on intrinsic excitability across brain regions, using a variety of behavioural paradigms. Most of them report an increase in intrinsic excitability following learning (Moyer et al., 1996; Thompson et al., 1996; Saar et al., 1998; Oh et al., 2003; Kuo et al., 2008; Matthews et al., 2008) including fear conditioning and extinction (Santini et al., 2008; McKay et al., 2009; Song et al., 2012; Sehgal et al., 2014; Senn et al., 2014). This is usually manifested by a reduction in the post-burst AHP and/or increased AP firing rates in response to depolarising current injections. In our study we find a change in intrinsic properties following learning. However, given our data it is not possible to conclude whether these changes represent a clear increase or a decrease in excitability. The depolarised RMP and increase in AP half width are typically associated with an increase in intrinsic excitability. On the other hand, a slower maximum *dV/dt* and increased post-burst AHP are indicators of decreased excitability. Finally, we observe no change in the overall number of AP fired in response to depolarising current pulses, which is the parameter most frequently associated with directional changes in neuronal excitability. The apparent lack of increase in excitability that we observed is therefore surprising and reconciling our results with previous research presents something of a challenge.

One possible reason for the discrepancies could be that the rules governing learning in the PL are different to those for the hippocampus (Moyer et al., 1996; Thompson et al., 1996; Oh et al., 2003; Kuo et al., 2008; Matthews et al., 2008; McKay et al., 2009), amygdala (Sehgal et al., 2014; Senn et al., 2014) or IL (Santini et al., 2008). To our knowledge only two accounts exist where the intrinsic excitability of PL neurons following fear conditioning has been investigated. The first one by Santini et al. (2008) suggested that fear conditioning does not alter the excitability of PL neurons. However, this is surprising given the well-established role of the PL in fear learning and expression (Vidal-Gonzalez et al., 2006; Burgos-Robles, 2009; Choi et al., 2010; Sotres-Bayon and Quirk, 2010; Sierra-Mercado et al., 2011; Courtin et al., 2013). The second account took a similar approach to ours and investigated intrinsic excitability specifically in the BLA-projecting PL neurons. This unpublished study by Song et al. (2013) showed that auditory trace fear conditioning suppresses the excitability of BLA-projecting PL neurons by reducing the number of APs evoked by depolarising current steps. Given that we observed no change in neuronal firing, it implies that our observations do not fully match those reported by Song et al., (2013). However, the apparent lack of increase in excitability that we both observe indicates that the rules governing learning can differ significantly between brain regions. Moreover, by comparing our results to those reported by Santini et al., (2008) it becomes apparent that a given brain region should not be treated as a whole and instead different neuronal populations within a single region could be differently altered by learning. The importance of targeting specific neuronal populations when investigating learning induced alterations in neuronal activity is being noticed. Recent studies have found that depending on their long-range connectivity, distinct neuronal populations in the BLA are differently activated by fear conditioning and extinction (Knapska et al., 2012; Senn et al., 2014).

Another reason for the discrepancies between our results and previous studies could be due to the exact behavioural protocol used. The majority of studies investigating learning induced changes in intrinsic excitability have used auditory fear conditioning protocols (Santini et al., 2008; McKay et al., 2009; Song et al., 2012; Song et al., 2013; Sehgal et al., 2014; Senn et al., 2014). However, fear learning in paradigms were the footshock is paired with a tone could occur on different rules to the one in which the presentation of the shock is unsignalled. Being able to distinguish between two different protocols could be of particular relevance to the PL. Indeed it is known that neurons in the PL show sustained responses to the conditioned tone (Burgos-Robles et al., 2009) as well as receive monosynaptic inputs from

the hippocampus (McDonald, 1991; Gabbott et a., 2002, 2006; Hoover and Vertes, 2007) likely conveying contextual information (Euston et al., 2012; Tronson et al., 2012). The fact that the PL recruites different neuronal mechanisms to process various fear conditioning paradigms has been demonstrated by Gilmartin & Helmstetter (2010). They showed that NMDA (N-methyl-D-aspartate) receptor mediated transmission is necessary for some but not all forms of contextual fear learning (Gilmartin & Helmstetter, 2010). The inhibition of PL activity with the GABAergic (γ-aminobutyric acid) agonist muscimol or the blockade of NMDA receptor mediated signalling with APV impairs the acquisition of contextual fear memory in an auditory fear conditioning paradigm. However, the memory impairment is only present when the tone is paired with the footshock. In contrast, when the tone is presented but it is not paired with the shock, the infusion of muscimol or APV has no effect on contextual fear acquisition. This unexpected finding shows that mechanisms supporting contextual fear conditioning are not the same across all training paradigms (Gilmartin & Helmstetter, 2010). Finally, correlating neuronal activity with learning in a contextual fear conditioning paradigm might be more challenging than for auditory fear conditioning. This could be due to the fact that the context is less distinct as a CS than a tone (Maren et al., 2013). The specific nature of the changes in intrinsic properties could therefore be more subtle/complex.

7.6 Implications of changes in intrinsic excitability

The study of intrinsic plasticity is particularly useful in the context of learning and memory because it can affect a wide range of neuronal functions including the presynaptic release of neurotransmitters, the way synaptic inputs are integrated, the AP output at the soma and the active back-flow of information into the dendrites. Furthermore, it could act as a regulator of synaptic plasticity, support rule learning, or in some cases serve as part of the memory trace itself (Zhang and Linden, 2003; Frick et al., 2004; Frick and Johnston, 2005; Disterhoft and Oh, 2006; Mozzachiodi and Byrne, 2010; Szlapczynska et al., 2014). The functional consequences of intrinsic plasticity for memory formation will depend on the nature of the change (e.g. increase or decrease in excitability), its cellular localization (e.g. neuron-wide or global versus individual dendritic branch), or its time course (transient, long-term). Several learning paradigms (including trace eyelid conditioning, olfactory discrimination, and fear conditioning) have been used to demonstrate a neuron-wide change in intrinsic excitability (Moyer et al., 1996; Saar et al., 1998; Kuo et al., 2008; McKay et al., 2009; Oh et al., 2009;

Sehgal et al., 2014). The significance of a global increase in neuronal excitability is not clear, but it may promote memory consolidation by (i) reducing the threshold for the induction of other forms of plasticity such as synaptic or structural plasticity, (ii) enhancing the likelihood that these neurons will be engaged in memory encoding, or (iii) enabling the selective re-activation of these neurons during post-training rest/sleep (Zhang and Linden, 2003; Frick and Johnston, 2005; Disterhoft and Oh, 2006; Euston et al., 2007; Peyrache et al., 2009; Zhou et al., 2009; Benchenane et al., 2010; Mozzachiodi and Byrne, 2010; Popa et al., 2010; Szlapczynska et al., 2014). The fact that an increase in intrinsic excitability could for example promote memory allocation to specific neurons is an interesting concept. Zhou et al., (2009) experimentally manipulated levels of the cellular transcription factor CREB (cyclic adenosine 3′,5′-monophosphate response element binding protein) in lateral amygdala (LA) neurons. They found that neurons expressing higher levels of CREB were more excitable and because of this they were more likely to get activated during fear conditioning and be recruited into storing the conditioning episode (Zhou et al., 2009).

Intrinsic plasticity is also closely coupled to synaptic plasticity and has been suggested to act as a possible substrate for metaplasticity (Frick and Johnston, 2005; Chen et al., 2006). Metaplasticity is a phenomenon whereby the neuron's ability to undergo synaptic plasticity is subject to the character of previously induced neuronal changes (Abraham and Bear, 1996). Therefore any long-term change in intrinsic properties that occurs as a consequence of behavioural learning or neuronal activity could in turn modulate the rules governing the induction of synaptic plasticity (Johnston et al., 2003; Sjöström et al., 2008). Indeed, activity induced alteration in the voltage-gated ion channel function is bound to influence the manner in which future synaptic inputs are integrated (Cash & Yuste, 1999; Magee, 2000; Williams and Stuart, 2003b; Magee & Johnston, 2005). Moreover, when backpropagating AP are paired with local EPSPs, this can results in increased Ca^{2+} influx the induction of NMDA receptor dependent LTP (Magee and Johnston, 1997; Waters et al., 2003; Letzkus et al., 2006; Sjöström and Häusser, 2006; Sjöström et al., 2008). Interestingly, depending on the exact timing of the backpropagation APs and the location of the activated synapses, the backpropagating AP can act as a bidirectional switch promoting the induction of either LTP or LTD (Letzkus et al., 2006; Sjöström and Häusser, 2006). Finally, short trains of backpropagationg APs have also been shown to cause an enduring depression of R-type Ca^{2+} channels in individual dendritic spines, which in turn inhibits the induction of synaptic plasticity (Yasuda et al., 2003).

Another possible function of intrinsic plasticity is the homeostatic regulation of neuronal activity (Turrigiano et al., 1994; Desai et al., 1999; Pratt and Aizenman, 2007; Grubb and Burrone, 2010; O'Leary et al., 2010). It could serve as a feedback mechanism that would allow the network to remain stable following for example Hebbian plasticity or acute changes in neuronal excitability (Aizenman and Linden, 2000; Frick et al., 2004; van Welie et al., 2004; Narayanan and Johnston, 2007; Brown and Randall, 2009). Intrinsic plasticity is well suited for this role because it plays a part in the regulation of neuronal firing rates (Frick & Johnston, 2005). Indeed, it has been shown that sustained alterations in neuronal activity can alter the expression of voltage-gated ion channels responsible for shaping firing patterns. For example, the long-term blocking (several days) of neuronal activity in cultured cortical pyramidal neurons can result in the upregulation of Na^+ channels and a downregulation of sustained K^+ currents. This in turn results in a lower threshold for AP initiation as well as increased firing frequency upon stimulation (Desai et al., 1999; Aptowicz et al., 2004). Another example comes from the studies on crustacean models. The lobster stomatogastric ganglion (STG) neurons fire in bursts upon synaptic and modulatory stimulation but fire tonically when pharmacologically isolated. However, 3-4 days of isolation of these neurons in culture results in a change of their firing pattern from tonic to bursting upon depolarisation. This is a consequence of an increase in Ca^{2+} and decrease in K^+ conductances that compensate for the absence of their normal synaptic inputs (Turrigiano et al., 1994; 1995). In contrast, exposing cultured hippocampal neurons to periods of sustained depolarisation by using a medium containing high KCl levels, results in a hyperpolarising shift in the RMP direction. Interestingly, the shift in the RMP becomes more hyperpolarized as KCl treatment concentration is increased. Importantly, however, when these cells are then reexposed to high KCl conditions they compensate by depolarising less than would be predicted for untreated cells (O'Leary et al., 2010). Blocking neuronal activity with glutamatergic antagonists CNQX and APV also results in a homeostatic increase in excitability through an increase in the number of AP. This effect is bidirectional because treatment with bicuculline - The $GABA_A$ receptor antagonist causes a decrease in neuronal excitability (Karmarkar and Buonomano, 2006).

7.7 Conclusions and future perspectives

Overall our study provides further evidence for learning-induced changes in intrinsic excitability. We extend previous findings by showing that fear conditioning induces intrinsic plasticity in the PL in a neuronal population specific manner. Moreover, our results add to the growing evidence for the importance of the PL-BLA pathway in fear memory processing. The exact direction of the observed changes, namely a decrease vs increase in excitability, could however not be concluded. This could have been due to the type of conditioning protocol used or the brain region/neuronal population under investigation. However, the specific pattern of changes that we observed could be interpreted in the light of feedback mechanisms employed by the neurons to prevent overexcitability and/or regulate the induction of subsequent synaptic plasticity by keeping previous associative plasticity intact. Moreover, our results could add to the understanding of the importance of more subtle and analogue forms of communication between neurons.

Given our findings, an important question arises: how does a change in intrinsic excitability translate into behaviour? One way to address this question would be to use optogenetic tools to experimentally control the activity of BLA-projecting PL neurons. This could be achieved by using a combination of viral tools with retrograde labelling to express light-sensitive microbial opsins in the specific neuronal population of interest (Zhang et al., 2010, 2011, Luo et al., 2008; Johansen et al., 2012; Madisen et al., 2012; Packer et al., 2013). The exact choice of these light-sensitive proteins would then allow for a bi-directional control of the cells' activity. For example, neurons expressing the depolarising cation channel Channelrhodopsin-2 (ChR2) in their membrane would be activated by light of an appropriate wavelength, whereas those expressing the hyperpolarizing proton pump Archaerhodopsin (Arch) would instead be inhibited (Chow et al., 2010; Zhang et al., 2010). In the context of our study ChR2 or Arch expressing BLA-projecting neurons could then be activated/inhibited during contextual fear memory retrieval. If the activation/inhibition of these neurons were indeed necessary for the expression of conditioned fear, an alteration in freezing level would be observable when compared to control animals.

Another direction for future research would be to use pharmacological approaches to better understand the channels mediating the learning-induced changes in excitability that we observed. Because we found a reduction in the maximum *dV/dt*, an increase in AP half-width and an increase in the post-bust AHP, the most obvious candidates for investigation would include: i) Na^+ channels that mediate the depolarising phase of the AP, ii) BK and/or Kv4

channels which control AP repolarisation and iii) SK channels underlying the post-burst AHP (Bean, 2007; Sah & Faber, 2002). Moreover, by using drug infusions *in vivo* as well as genetic overexpression/knockout approaches it might be possible to elucidate the importance of these channels for mediating fear expression and memory.

Finally, because the time course of changes in intrinsic plasticity is most often limited to several days (Moyer Jr et al., 1996; Thompson et al., 1996; Saar et al., 1998) it would be interesting to investigate how long the changes that we observed persist for. Furthermore, as suggested earlier in this section, different memory phases might require the activation of different neuronal populations. For this reason, it would be interesting to investigate whether the intrinsic properties of mPFC-projecting neurons could be altered at more remote time points. In conclusion this work not only demonstrates that contextual fear conditioning induces neuronal population specific changes in intrinsic excitability, it also sets the stage for understanding the functional role of these changes for memory expression.

References

A

Abel, H.J., Lee, J.C.F., Callaway, J.C., and Foehring, R.C. (2004). Relationships between intracellular calcium and afterhyperpolarizations in neocortical pyramidal neurons. J. Neurophysiol. *91*, 324–335.

Abraham, W.C., and Bear, M.F. (1996). Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci *19*, 126–130.

Abraham, W.C., Gustafsson, B., and Wigström, H. (1987). Long-term potentiation involves enhanced synaptic excitation relative to synaptic inhibition in guinea-pig hippocampus. J. Physiol. *394*, 367–380.

Adhikari, A., Topiwala, M.A., and Gordon, J.A. (2010). Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. Neuron *65*, 257–269.

van Aerde, K.I., and Feldmeyer, D. (2013). Morphological and Physiological Characterization of Pyramidal Neuron Subtypes in Rat Medial Prefrontal Cortex. Cereb. Cortex. Advanced Online Access, Published 9 December 2013, doi:10.1093/cercor/bht278

Antonov, I., Antonova, I., Kandel, E.R., and Hawkins, R.D. (2001). The contribution of activity-dependent synaptic plasticity to classical conditioning in Aplysia. J. Neurosci. *21*, 6413–6422.

Aizenman, C.D., and Linden, D.J. (2000). Rapid, synaptically driven increases in the intrinsic excitability of cerebellar deep nuclear neurons. Nat. Neurosci. *3*, 109–111.

Akirav, I., and Maroun, M. (2005). Ventromedial Prefrontal Cortex Is Obligatory for Consolidation and Reconsolidation of Object Recognition Memory. Cereb. Cortex *16*, 1759–1765.

Alkon, D.L., Farley, J., Sakakibara, M., and Hay, B. (1984). Voltage-dependent calcium and calcium-activated potassium currents of a molluscan photoreceptor. Biophys. J. *46*, 605–614.

Alkon, D.L., Lederhendler, I., and Shoukimas, J.J. (1982). Primary changes of membrane currents during retention of associative learning. Science *215*, 693–695.

Alkon, D.L., Sakakibara, M., Forman, R., Harrigan, J., Lederhendler, I., and Farley, J. (1985). Reduction of two voltage-dependent K+ currents mediates retention of a learned association. Behav. Neural Biol. *44*, 278–300.

Alle, H., and Geiger, J.R.P. (2006). Combined analog and action potential coding in hippocampal mossy fibers. Science *311*, 1290–1293.

Andrade, R., Foehring, R.C., and Tzingounis, A.V. (2012). The calcium-activated slow AHP: cutting through the Gordian knot. Front Cell Neurosci. *6*, 47.

Amaral, D.G. and Lavenex, P. (2007). Hippocampal neuroanatomy. In The Hippocampus Book, P. Andersen, R. Morris, D. Amaral, T. Bliss and J. O'Keefe, eds. (Oxford: Oxford University Press), pp. 37–131.

Amaral, D.G. and Witter, M.P. (1995) Hippocampal formation. In The Rat Nervous System, G. Paxinos, ed. (San Diego, CA: Acad. Press Inc.), pp. 443-493.

Anagnostaras, S.G., Josselyn, S.A., Frankland, P.W., and Silva, A.J. (2000). Computer-assisted behavioral assessment of Pavlovian fear conditioning in mice. Learn. Memory *7*, 58–72.

Anagnostaras, S.G., Maren, S., and Fanselow, M.S. (1999). Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. J. Neurosci. *19*, 1106–1114.

Applegate, C.D., Frysinger, R.C., Kapp, B.S., and Gallagher, M. (1982). Multiple unit activity recorded from amygdala central nucleus during Pavlovian heart rate conditioning in rabbit. Brain Res. *238*, 457–462.

Aptowicz, C.O., Kunkler, P.E., and Kraig, R.P. (2004). Homeostatic plasticity in hippocampal slice cultures involves changes in voltage-gated Na+ channel expression. Brain Res. *998*, 155–163.

B

Bacon, S.J., Headlam, A.J., Gabbott, P.L., and Smith, A.D. (1996). Amygdala input to medial prefrontal cortex (mPFC) in the rat: a light and electron microscope study. Brain Res. *720*, 211–219.

Baddeley, A.D. (2001). The concept of episodic memory. Philos. Trans. R. Soc. London [Biol.] *356*, 1345–1350.

Baddeley, A.D. and Hitch, G.J. (1974). Working memory. In Recent Advances in Learning and Motivation, Vol. 8, G.A. Bower, ed. (New York: Academic Press), pp. 47–89.

Baeg, E.H., Kim, Y.B., Jang, J., Kim, H.T., Mook-Jung, I., and Jung, M.W. (2001). Fast spiking and regular spiking neural correlates of fear conditioning in the medial prefrontal cortex of the rat. Cereb. Cortex *11*, 441–451.

Bauer, E.P., Schafe, G.E., and LeDoux, J.E. (2002). NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. J. Neurosci. *22*, 5239–5249.

Bayley, P.J., Gold, J.J., Hopkins, R.O., and Squire, L.R. (2005). The Neuroanatomy of Remote Memory. Neuron *46*, 799–810.

Bean, B.P. (2007). The action potential in mammalian central neurons. Nat. Rev. Neurosci. *8*, 451–465.

Bechara, A., and Damasio, A.R. (2005). The somatic marker hypothesis: A neural theory of economic decision. Games Econ. Behav. 52, 336–372.

Bekkers, J.M., and Delaney, A.J. (2001). Modulation of excitability by alpha-dendrotoxin-sensitive potassium channels in neocortical pyramidal neurons. J. Neurosci. *21*, 6553–6560.

Benchenane, K., Peyrache, A., Khamassi, M., Tierney, P.L., Gioanni, Y., Battaglia, F.P., and Wiener, S.I. (2010). Coherent Theta Oscillations and Reorganization of Spike Timing in the Hippocampal- Prefrontal Network upon Learning. Neuron *66*, 921–936.

Benchenane, K., Tiesinga, P.H., and Battaglia, F.P. (2011). Oscillations in the prefrontal cortex: a gateway to memory and attention. Curr. Opin. Neurobiol. *21*, 475–485.

Beck, C.H., and Fibiger, H.C. (1995). Conditioned fear-induced changes in behavior and in the expression of the immediate early gene c-fos: with and without diazepam pretreatment. J. Neurosci. *15*, 709–720.

Benhassine, N., and Berger, T. (2005). Homogeneous distribution of large-conductance calcium-dependent potassium channels on soma and apical dendrite of rat neocortical layer 5 pyramidal neurons. Eur. J. Neurosci. *21*, 914–926.

Benito, E., and Barco, A. (2010). CREB's control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. Trends Neurosci. *33*, 230–240.

Berger, T., Larkum, M.E., and Lüscher, H.R. (2001). High I(h) channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. J. Neurophysiol. *85*, 855–868.

Bissière, S., Plachta, N., Hoyer, D., McAllister, K.H., Olpe, H.-R., Grace, A.A., and Cryan, J.F. (2008). The Rostral Anterior Cingulate Cortex Modulates the Efficiency of Amygdala-Dependent Fear Learning. Biol. Psychiatry *63*, 821–831.

Blair, H.T. (2001). Synaptic Plasticity in the Lateral Amygdala: A Cellular Hypothesis of Fear Conditioning. Learn. Memory *8*, 229–242.

Blanchard, D.C., and Blanchard, R.J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. J. Comp. Physiol. Psychol. *81*, 281–290.

Bliss, T.V., and Collingridge, G.L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature *361*, 31–39.

Bliss, T.V., and Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J. Physiol. *232*, 331–356.

Blum, S., Hebert, A.E., and Dash, P.K. (2006). A role for the prefrontal cortex in recall of recent and remote memories. Neuroreport *17*, 341–344.

Bontempi, B., Laurent-Demir, C., Destrade, C., and Jaffard, R. (1999). Time-dependent reorganization of brain circuitry underlying long-term memory storage. Nature *400*, 671–675.

Bordi, F., and LeDoux, J.E. (1994). Response properties of single units in areas of rat auditory thalamus that project to the amygdala. II. Cells receiving convergent auditory and somatosensory inputs and cells antidromically activated by amygdala stimulation. Exp. Brain Res. *98*, 275–286.

Botvinick, M.M., Cohen, J.D., and Carter, C.S. (2004). Conflict monitoring and anterior cingulate cortex: an update. Trends in Cognitive Sciences *8*, 539–546.

Brennan, A.R., Dolinsky, B., Vu, M.A.T., Stanley, M., Yeckel, M.F., and Arnsten, A.F.T. (2008). Blockade of IP3-mediated SK channel signaling in the rat medial prefrontal cortex improves spatial working memory. Learn. Memory *15*, 93–96.

Breton, J.D., and Stuart, G.J. (2009). Loss of sensory input increases the intrinsic excitability of layer 5 pyramidal neurons in rat barrel cortex. J. Physiol. *587*, 5107–5119.

Brons, J.F., and Woody, C.D. (1980). Long-term changes in excitability of cortical neurons after Pavlovian conditioning and extinction. J. Neurophysiol. *44*, 605–615.

Brown, V.J., and Bowman, E.M. (2002). Rodent models of prefrontal cortical function. Trends Neurosci. *25*, 340–343.

Brown, D.A., and Passmore, G.M. (2009). Neural KCNQ (Kv7) channels. Br. J. Pharmacol. *156*, 1185–1195.

Brown, J.T., and Randall, A.D. (2009). Activity-dependent depression of the spike after-depolarization generates long-lasting intrinsic plasticity in hippocampal CA3 pyramidal neurons. J. Physiol. *587*, 1265–1281.

Burgos-Robles, A., Vidal-Gonzalez, I., and Quirk, G.J. (2009). Sustained Conditioned Responses in Prelimbic Prefrontal Neurons Are Correlated with Fear Expression and Extinction Failure. J. Neurosci. *29*, 8474–8482.

Burgos-Robles, A., Vidal-Gonzalez, I., Santini, E., and Quirk, G.J. (2007). Consolidation of Fear Extinction Requires NMDA Receptor-Dependent Bursting in the Ventromedial Prefrontal Cortex. Neuron *53*, 871–880.

Buzsáki, G. (2004). Neuronal Oscillations in Cortical Networks. Science *304*, 1926–1929.

C

Cai, X., Liang, C.W., Muralidharan, S., Kao, J.P.Y., Tang, C.-M., and Thompson, S.M. (2004). Unique Roles of SK and Kv4.2 Potassium Channels in Dendritic Integration. Neuron *44*, 351–364.

Canteras, N.S., and Swanson, L.W. (1992). Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: a PHAL anterograde tract-tracing study in the rat. J. Comp. Neurol. *324*, 180–194.

Carballo-Márquez, A., Vale-Martínez, A., Guillazo-Blanch, G., and Martí-Nicolovius, M. (2009). Muscarinic receptor blockade in ventral hippocampus and prelimbic cortex impairs memory for socially transmitted food preference. Hippocampus *19*, 446–455.

Carballo-Marquez, A., Vale-Martinez, A., Guillazo-Blanch, G., Torras-Garcia, M., Boix-Trelis, N., and Marti-Nicolovius, M. (2007). Differential effects of muscarinic receptor blockade in prelimbic cortex on acquisition and memory formation of an odor-reward task. Learn. Memory *14*, 616–624.

Cash, S., and Yuste, R. (1999). Linear summation of excitatory inputs by CA1 pyramidal neurons. Neuron *22*, 383–394.

Cassell, M.D., Gray, T.S., and Kiss, J.Z. (1986). Neuronal architecture in the rat central nucleus of the amygdala: a cytological, hodological, and immunocytochemical study. J. Comp. Neurol. *246*, 478–499.

Catterall, W.A. (2000). Structure and regulation of voltage-gated Ca2+ channels. Annu. Rev. Cell Dev. Biol. *16*, 521–555.

Catterall, W.A. (2011). Voltage-gated calcium channels. Cold Spring Harb Perspect Biol *3*, a003947.

Cantrell, A.R., and Catterall, W.A. (2001). Neuromodulation of Na+ channels: an unexpected form of cellular plasticity. Nat. Rev. Neurosci. *2*, 397–407.

Catterall, W.A., Raman, I.M., Robinson, H.P.C., Sejnowski, T.J., and Paulsen, O. (2012). The Hodgkin-Huxley Heritage: From Channels to Circuits. J. Neurosci. *32*, 14064–14073.

Chavez-Noriega, L.E., Bliss, T.V., and Halliwell, J.V. (1989). The EPSP-spike (E-S) component of long-term potentiation in the rat hippocampal slice is modulated by GABAergic but not cholinergic mechanisms. Neurosci. Lett. *104*, 58–64.

Chen, X., Yuan, L.-L., Zhao, C., Birnbaum, S.G., Frick, A., Jung, W.E., Schwarz, T.L., Sweatt, J.D., and Johnston, D. (2006). Deletion of Kv4.2 gene eliminates dendritic A-type K+ current and enhances induction of long-term potentiation in hippocampal CA1 pyramidal neurons. J. Neurosci. *26*, 12143–12151.

Cheung, T.H.C., and Cardinal, R.N. (2005). Hippocampal lesions facilitate instrumental learning with delayed reinforcement but induce impulsive choice in rats. BMC Neurosci. *6*, 36.

Cho, Y.H., Friedman, E., and Silva, A.J. (1999). Ibotenate lesions of the hippocampus impair spatial learning but not contextual fear conditioning in mice. Behav. Brain Res. *98*, 77–87.

Choi, D.C., Maguschak, K.A., Ye, K., Jang, S.W., Myers, K.M., and Ressler, K.J. (2010). Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. Proc. Natl. Acad. Sci. USA *107*, 2675–2680.

Chow, B.Y., Han, X., Dobry, A.S., Qian, X., Chuong, A.S., Li, M., Henninger, M.A., Belfort, G.M., Lin, Y., Monahan, P.E., et al. (2010). High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature *463*, 98–102.

Churchwell, J.C., Morris, A.M., Musso, N.D., and Kesner, R.P. (2010). Prefrontal and hippocampal contributions to encoding and retrieval of spatial memory. Neurobiol. Lear. Mem. *93*, 415–421.

Ciocchi, S., Herry, C., Grenier, F., Wolff, S.B.E., Letzkus, J.J., Vlachos, I., Ehrlich, I., Sprengel, R., Deisseroth, K., Stadler, M.B., et al. (2010). Encoding of conditioned fear in central amygdala inhibitory circuits. Nature *468*, 277–282.

Cleary, L.J., Lee, W.L., and Byrne, J.H. (1998). Cellular correlates of long-term sensitization in Aplysia. J. Neurosci. *18*, 5988–5998.

Clugnet, M.C., and LeDoux, J.E. (1990). Synaptic plasticity in fear conditioning circuits: induction of LTP in the lateral nucleus of the amygdala by stimulation of the medial geniculate body. J. Neurosci. *10*, 2818–2824.

Colbert, C.M., Magee, J.C., Hoffman, D.A., and Johnston, D. (1997). Slow recovery from inactivation of Na+ channels underlies the activity-dependent attenuation of dendritic action potentials in hippocampal CA1 pyramidal neurons. J. Neurosci. *17*, 6512–6521.

Corcoran, K.A., and Quirk, G.J. (2007). Activity in Prelimbic Cortex Is Necessary for the Expression of Learned, But Not Innate, Fears. J. Neurosci. *27*, 840–844.

Courtin, J., Bienvenu, T.C.M., Einarsson, E.O., and Herry, C. (2013). Medial prefrontal cortex neuronal circuits in fear behavior. Neuroscience *240*, 219–242.

Courtin, J., Chaudun, F., Rozeske, R.R., Karalis, N., Gonzalez-Campo, C., Wurtz, H., Abdi, A., Baufreton, J., Bienvenu, T.C.M., and Herry, C. (2014). Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression. Nature *505*, 92–96.

Criado-Marrero, M., Santini, E., and Porter, J.T. (2014). Modulating fear extinction memory by manipulating SK potassium channels in the infralimbic cortex. Front. Behav. Neurosci. *8*, 96.

Crill, W.E. (1996). Persistent sodium current in mammalian central neurons. Annu. Rev. Physiol. *58*, 349–362.

D

Dan, Y., and Poo, M.M. (2006). Spike timing-dependent plasticity: from synapse to perception. Physiol. Rev. *86*, 1033–1048.

Daoudal, G., Hanada, Y., and Debanne, D. (2002). Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. Proc. Natl. Acad. Sci. USA *99*, 14512–14517.

Davis, M. (1992). The role of the amygdala in fear and anxiety. Annu. Rev. Neurosci. *15*, 353–375.

Debanne, D. (2004). Information processing in the axon. Nat. Rev. Neurosci. *5*, 304–316.

Debanne, D., Campanac, E., Bialowas, A., Carlier, E., and Alcaraz, G. (2011). Axon Physiology. Physiol. Rev. *91*, 555–602.

Dembrow, N.C., Chitwood, R.A., and Johnston, D. (2010). Projection-Specific Neuromodulation of Medial Prefrontal Cortex Neurons. J. Neurosci. *30*, 16922–16937.

Deng, P.-Y., Rotman, Z., Blundon, J.A., Cho, Y., Cui, J., Cavalli, V., Zakharenko, S.S., and Klyachko, V.A. (2013). FMRP Regulates Neurotransmitter Release and Synaptic Information Transmission by Modulating Action Potential Duration via BK Channels. Neuron *77*, 696–711.

Desai, N.S., Rutherford, L.C., and Turrigiano, G.G. (1999). Plasticity in the intrinsic excitability of cortical pyramidal neurons. Nat. Neurosci. *2*, 515–520.

Deschaux, O., Bizot, J.C., and Goyffon, M. (1997). Apamin improves learning in an object recognition task in rats. Neurosci. Lett. *222*, 159–162.

Ding, H.K., Teixeira, C.M., and Frankland, P.W. (2008). Inactivation of the anterior cingulate cortex blocks expression of remote, but not recent, conditioned taste aversion memory. Learn. Memory *15*, 290–293.

Disterhoft, J.F., and Oh, M.M. (2006). Learning, aging and intrinsic neuronal plasticity. Trends Neurosci. *29*, 587–599.

Disterhoft, J.F., Wu, W.W., and Ohno, M. (2004). Biophysical alterations of hippocampal pyramidal neurons in learning, ageing and Alzheimer's disease. Ageing Research Reviews *3*, 383–406.

Dodt, H.U., and Zieglgänsberger, W. (1990). Visualizing unstained neurons in living brain slices by infrared DIC-videomicroscopy. Br. Res. *537*, 333–336.

Dougherty, K.A., Islam, T., and Johnston, D. (2012). Intrinsic excitability of CA1 pyramidal neurones from the rat dorsal and ventral hippocampus. J. Physiol. *590*, 5707–5722.

Douglas, R.J., and Martin, K.A.C. (2004). Neuronal circuits of the neocortex. Annu. Rev. Neurosci. *27*, 419–451.

E

Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C., and Lüthi, A. (2009). Amygdala Inhibitory Circuits and the Control of Fear Memory. Neuron *62*, 757–771.

Einarsson, E.O., and Nader, K. (2012). Involvement of the anterior cingulate cortex in formation, consolidation, and reconsolidation of recent and remote contextual fear memory. Learn. Memory *19*, 449–452.

Euston, D.R., Gruber, A.J., and McNaughton, B.L. (2012). The Role of Medial Prefrontal Cortex in Memory and Decision Making. Neuron *76*, 1057–1070.

Euston, D.R., Tatsuno, M., and McNaughton, B.L. (2007). Fast-Forward Playback of Recent Memory Sequences in Prefrontal Cortex During Sleep. Science *318*, 1147–1150.

Evans, M.D., Sammons, R.P., Lebron, S., Dumitrescu, A.S., Watkins, T.B.K., Uebele, V.N., Renger, J.J., and Grubb, M.S. (2013). Calcineurin Signaling Mediates Activity-Dependent Relocation of the Axon Initial Segment. J. Neurosci. *33*, 6950–6963.

F

Faber, E.S.L. (2010). Functional interplay between NMDA receptors, SK channels and voltage-gated Ca2+ channels regulates synaptic excitability in the medial prefrontal cortex. J. Physiol. *588*, 1281–1292.

Faber, E.S.L., and Sah, P. (2003). Ca2+-activated K+ (BK) channel inactivation contributes to spike broadening during repetitive firing in the rat lateral amygdala. J. Physiol. *552*, 483–497.

Faber, E.S.L., and Sah, P. (2007). Functions of SK channels in central neurons. Clin. Exp. Pharmacol. Physiol. *34*, 1077–1083.

Fanselow, M. S. (1986). Associative vs topographical accounts of the immediate shock-freezing deficit in rats: Implication for the response selection rules governing species-specific defensive reactions. *Learning and Motivation, 17,* 16–39.

Fanselow, M.S. (1990). Factors governing one-trial contextual conditioning. Learning & Behavior *18*, 264–270.

Fanselow, M.S. (2000). Contextual fear, gestalt memories, and the hippocampus. Behav. Brain Res. *110*, 73–81.

Fanselow, M.S. (2010). From contextual fear to a dynamic view of memory systems. Trends in Cognitive Sciences *14*, 7–15.

Fanselow, M.S., and Kim, J.J. (1994). Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. Behav. Neurosci. *108*, 210–212.

Feldmeyer, D. (2012). Excitatory neuronal connectivity in the barrel cortex. Front. Neuroanat. *6*, 24.

Feldmeyer, D., Lübke, J., Silver, R.A., and Sakmann, B. (2002). Synaptic connections between layer 4 spiny neurone-layer 2/3 pyramidal cell pairs in juvenile rat barrel cortex: physiology and anatomy of interlaminar signalling within a cortical column. J. Physiol. *538*, 803–822.

Fenton, G.E., Pollard, A.K., Halliday, D.M., Mason, R., Bredy, T.W., and Stevenson, C.W. (2014). Persistent prelimbic cortex activity contributes to enhanced learned fear expression in females. Learn. Memory *21*, 55–60.

Flavell, C.R., and Lee, J.L.C. (2012). Post-training unilateral amygdala lesions selectively impair contextual fear memories. Learn. Memory *19*, 256–263.

Foust, A.J., Yu, Y., Popovic, M., Zecevic, D., and McCormick, D.A. (2011). Somatic Membrane Potential and Kv1 Channels Control Spike Repolarization in Cortical Axon Collaterals and Presynaptic Boutons. J. Neurosci. *31*, 15490–15498.

Frankland, P.W., Bontempi, B., Talton, L.E., Kaczmarek, L., and Silva, A.J. (2004). The involvement of the anterior cingulate cortex in remote contextual fear memory. Science *304*, 881.

Frankland, P.W., Cestari, V., Filipkowski, R.K., McDonald, R.J., and Silva, A.J. (1998). The dorsal hippocampus is essential for context discrimination but not for contextual conditioning. Behav. Neurosci. *112*, 863–874.

Frankland, P.W., Ding, H.-K., Takahashi, E., Suzuki, A., Kida, S., and Silva, A.J. (2006). Stability of recent and remote contextual fear memory. Learn. Memory *13*, 451–457.

Frick, A., and Johnston, D. (2005). Plasticity of dendritic excitability. J. Neurobiol. *64*, 100–115.

Frick, A., Magee, J., and Johnston, D. (2004). LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. Nat. Neurosci. *7*, 126–135.

Frick, A., Magee, J., Koester, H.J., Migliore, M., and Johnston, D. (2003). Normalization of Ca2+ signals by small oblique dendrites of CA1 pyramidal neurons. J. Neurosci. *23*, 3243–3250.

Fuster, J. M. (2008). The Prefrontal Cortex, 4th Edn. (Boston, MA: Academic Press).

G

Gabbott, P.L.A., Dickie, B.G., Vaid, R.R., Headlam, A.J., and Bacon, S.J. (1997). Local-circuit neurones in the medial prefrontal cortex (areas 25, 32 and 24b) in the rat: morphology and quantitative distribution. J. Comp. Neurol. *377*, 465–499.

Gabbott, P., Headlam, A., and Busby, S. (2002). Morphological evidence that CA1 hippocampal afferents monosynaptically innervate PV-containing neurons and NADPH-diaphorase reactive cells in the medial prefrontal cortex (Areas 25/32) of the rat. Br. Res. *946*, 314–322.

Gabbott, P.L.A., Warner, T.A., and Busby, S.J. (2006). Amygdala input monosynaptically innervates parvalbumin immunoreactive local circuit neurons in rat medial prefrontal cortex. Neuroscience *139*, 1039–1048.

Gabbott, P., Warner, T.-A., Brown, J., Salway, P., Gabbott, T., and Busby, S. (2012). Amygdala afferents monosynaptically innervate corticospinal neurons in rat medial prefrontal cortex. J. Comp. Neurol. *520*, 2440–2458.

Gabbott, P.L.A., Warner, T.A., Jays, P.R.L., and Bacon, S.J. (2003). Areal and synaptic interconnectivity of prelimbic (area 32), infralimbic (area 25) and insular cortices in the rat. Brain Research *993*, 59–71.

Gabbott, P.L.A., Warner, T.A., Jays, P.R.L., Salway, P., and Busby, S.J. (2005). Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. J. Comp. Neurol. *492*, 145–177.

Gainutdinov, K.L., Chekmarev, L.J., and Gainutdinova, T.H. (1998). Excitability increase in withdrawal interneurons after conditioning in snail. Neuroreport *9*, 517–520.

Gale, G.D., Anagnostaras, S.G., Godsil, B.P., Mitchell, S., Nozawa, T., Sage, J.R., Wiltgen, B., and Fanselow, M.S. (2004). Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats. J. Neurosci. *24*, 3810–3815.

Geiger, J.R., and Jonas, P. (2000). Dynamic control of presynaptic Ca(2+) inflow by fast-inactivating K(+) channels in hippocampal mossy fiber boutons. Neuron *28*, 927–939.

Gentet, L.J., and Williams, S.R. (2007). Dopamine gates action potential backpropagation in midbrain dopaminergic neurons. J. Neurosci. *27*, 1892–1901.

Gilmartin, M.R., and Helmstetter, F.J. (2010). Trace and contextual fear conditioning require neural activity and NMDA receptor-dependent transmission in the medial prefrontal cortex. Learn. Memory *17*, 289–296.

Gilmartin, M.R., and McEchron, M.D. (2005). Single neurons in the medial prefrontal cortex of the rat exhibit tonic and phasic coding during trace fear conditioning. Behav. Neurosci. 119, 1496–1510.

Golding, N.L., Kath, W.L., and Spruston, N. (2001). Dichotomy of action-potential backpropagation in CA1 pyramidal neuron dendrites. J. Neurophysiol. *86*, 2998–3010.

Goosens, K.A., Hobin, J.A., and Maren, S. (2003). Auditory-evoked spike firing in the lateral amygdala and Pavlovian fear conditioning: mnemonic code or fear bias? Neuron *40*, 1013–1022.

Goosens, K.A., and Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. Learn. Memory *8*, 148–155.

Grewe, B.F., Bonnan, A., and Frick, A. (2010). Back-Propagation of Physiological Action Potential Output in Dendrites of Slender-Tufted L5A Pyramidal Neurons. Front Cell Neurosci. *4*, 13.

Grubb, M.S., and Burrone, J. (2010). Activity-dependent relocation of the axon initial segment fine-tunes neuronal excitability. Nature *465*, 1070–1074.

Guan, D., Armstrong, W.E., and Foehring, R.C. (2013). Kv2 channels regulate firing rate in pyramidal neurons from rat sensorimotor cortex. J. Physiol. *591*, 4807–4825.

Guan, D., Higgs, M.H., Horton, L.R., Spain, W.J., and Foehring, R.C. (2011). Contributions of Kv7-mediated potassium current to sub- and suprathreshold responses of rat layer II/III neocortical pyramidal neurons. J. Neurophysiol. *106*, 1722–1733.

Guan, D., Lee, J.C.F., Higgs, M.H., Spain, W.J., and Foehring, R.C. (2007a). Functional roles of Kv1 channels in neocortical pyramidal neurons. J. Neurophysiol. *97*, 1931–1940.

Guan, D., Tkatch, T., Surmeier, D.J., Armstrong, W.E., and Foehring, R.C. (2007b). Kv2 subunits underlie slowly inactivating potassium current in rat neocortical pyramidal neurons. J. Physiol. *581*, 941–960.

Gulledge, A.T., Kampa, B.M., and Stuart, G.J. (2005). Synaptic integration in dendritic trees. J. Neurobiol. *64*, 75–90.

Gulledge, A.T., Park, S.B., Kawaguchi, Y., and Stuart, G.J. (2007). Heterogeneity of Phasic Cholinergic Signaling in Neocortical Neurons. J. Neurophysiol. *97*, 2215–2229.

Gutman, G.A., Chandy, K.G., Grissmer, S., Lazdunski, M., McKinnon, D., Pardo, L.A., Robertson, G.A., Rudy, B., Sanguinetti, M.C., Stühmer, W., et al. (2005). International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. Pharmacol. Rev. *57*, 473–508.

H

Hagenston, A.M., Fitzpatrick, J.S., and Yeckel, M.F. (2008). MGluR-Mediated Calcium Waves that Invade the Soma Regulate Firing in Layer V Medial Prefrontal Cortical Pyramidal Neurons. Cereb. Cortex *18*, 407–423.

Hammond, R.S., Bond, C.T., Strassmaier, T., Ngo-Anh, T.J., Adelman, J.P., Maylie, J., and Stackman, R.W. (2006). Small-conductance Ca2+-activated K+ channel type 2 (SK2) modulates hippocampal learning, memory, and synaptic plasticity. J. Neurosci. *26*, 1844–1853.

Haubensak, W., Kunwar, P.S., Cai, H., Ciocchi, S., Wall, N.R., Ponnusamy, R., Biag, J., Dong, H.-W., Deisseroth, K., Callaway, E.M., et al. (2010). Genetic dissection of an amygdala microcircuit that gates conditioned fear. Nature *468*, 270–276.

Hausser, M., Major, G., and Stuart, G.J. (2001). Differential shunting of EPSPs by action potentials. Science *291*, 138–141.

Hausser, M., Spruston, N., and Stuart, G.J. (2000). Diversity and dynamics of dendritic signaling. Science *290*, 739–744.

Hebb, D.O. (1949). The Organization of Behavior: A Neuropsychological Theory. (New York: Wiley and Sons).

Hefner, K., Whittle, N., Juhasz, J., Norcross, M., Karlsson, R.M., Saksida, L.M., Bussey, T.J., Singewald, N., and Holmes, A. (2008). Impaired Fear Extinction Learning and Cortico-Amygdala Circuit Abnormalities in a Common Genetic Mouse Strain. J. Neurosci. *28*, 8074–8085.

Heidbreder, C.A., and Groenewegen, H.J. (2003). The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neurosci. Biobehav. Rev. *27*, 555–579.

Helmchen, F., Imoto, K., and Sakmann, B. (1996). Ca2+ buffering and action potential-evoked Ca2+ signaling in dendrites of pyramidal neurons. Biophys. J. *70*, 1069–1081.

Helmchen, F., Svoboda, K., Denk, W., and Tank, D.W. (1999). In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons. Nat. Neurosci. *2*, 989–996.

Herry, C., Ciocchi, S., Senn, V., Demmou, L., Müller, C., and Lüthi, A. (2008). Switching on and off fear by distinct neuronal circuits. Nature *454*, 600–606.

Herry, C., Ferraguti, F., Singewald, N., Letzkus, J.J., Ehrlich, I., and Lüthi, A. (2010). Neuronal circuits of fear extinction. Eur. J. Neurosci. *31*, 599–612.

Herry, C., and Mons, N. (2004). Resistance to extinction is associated with impaired immediate early gene induction in medial prefrontal cortex and amygdala. Eur. J. Neurosci. *20*, 781–790.

Hikind, N., and Maroun, M. (2008). Microinfusion of the D1 receptor antagonist, SCH23390 into the IL but not the BLA impairs consolidation of extinction of auditory fear conditioning. Neurobiol. Learn. Mem. *90*, 217–222.

Hille, B. (2001). Ionic channels of excitable membranes (Sunderland, MA: Sinauer).

Hoffman, D.A., and Johnston, D. (1998). Downregulation of transient K+ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. J. Neurosci. *18*, 3521–3528.

Hoffman, D.A., and Johnston, D. (1999). Neuromodulation of dendritic action potentials. J. Neurophysiol. *81*, 408–411.

Hodgkin, A.L. and Huxley, A.F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol *117*, 500–544.

Holloway, C.M., and McIntyre, C.K. (2011). Post-training disruption of Arc protein expression in the anterior cingulate cortex impairs long-term memory for inhibitory avoidance training. Neurobiol. Learn. Mem. *95*, 425–432.

Holroyd, C.B., Coles, M.G., and Nieuwenhuis, S. (2002). Medial prefrontal cortex and error potentials. Science 296, 1610–1611.

Hoover, W.B., and Vertes, R.P. (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct. Funct. *212*, 149–179.

de Hoz, L., Moser, E.I., and Morris, R.G.M. (2005). Spatial learning with unilateral and bilateral hippocampal networks. Eur. J. Neurosci. *22*, 745–754.

Huff, N.C., Wright-Hardesty, K.J., Higgins, E.A., Matus-Amat, P., and Rudy, J.W. (2005). Context pre-exposure obscures amygdala modulation of contextual-fear conditioning. Learn. Memory *12*, 456–460.

Hyman, J.M., Ma, L., Balaguer-Ballester, E., Durstewitz, D., and Seamans, J.K. (2012). Contextual encoding by ensembles of medial prefrontal cortex neurons. Proc. Natl. Acad. Sci. USA *109*, 5086–5091.

Hyman, J.M., Zilli, E.A., Paley, A.M., and Hasselmo, M.E. (2005). Medial prefrontal cortex cells show dynamic modulation with the hippocampal theta rhythm dependent on behavior. Hippocampus *15*, 739–749.

J

Johansen, J.P., Cain, C.K., Ostroff, L.E., and LeDoux, J.E. (2011). Molecular Mechanisms of Fear Learning and Memory. Cell *147*, 509–524.

Johansen, J.P., Fields, H.L., and Manning, B.H. (2001). The affective compo- nent of pain in rodents: direct evidence for a contribution of the anterior cingu- late cortex. Proc. Natl. Acad. Sci. USA 98, 8077–8082.

Johansen, J.P., Wolff, S.B.E., Lüthi, A., and LeDoux, J.E. (2012). Controlling the elements: an optogenetic approach to understanding the neural circuits of fear. Biol. Psychiatry *71*, 1053–1060.

Johnston, D., Christie, B.R., Frick, A., Gray, R., Hoffman, D.A., Schexnayder, L.K., Watanabe, S., and Yuan, L.-L. (2003). Active dendrites, potassium channels and synaptic plasticity. Philos. Trans. R. Soc. Lond., B, Biol. Sci. *358*, 667–674.

Johnston, D., Magee, J.C., Colbert, C.M., and Cristie, B.R. (1996). Active properties of neuronal dendrites. Annu. Rev. Neurosci. *19*, 165–186.

Johnston, D., and Narayanan, R. (2008). Active dendrites: colorful wings of the mysterious butterflies. Trends Neurosci. *31*, 309–316.

Jones, M.W., and Wilson, M.A. (2005). Theta Rhythms Coordinate Hippocampal–Prefrontal Interactions in a Spatial Memory Task. Plos Biol. *3*, e402.

de Jonge, M.C., Black, J., Deyo, R.A., and Disterhoft, J.F. (1990). Learning-induced afterhyperpolarization reductions in hippocampus are specific for cell type and potassium conductance. Exp. Br. Res. *80*, 456–462.

Juusola, M., Robinson, H.P.C., and de Polavieja, G.G. (2007). Coding with spike shapes and graded potentials in cortical networks. Bioessays *29*, 178–187.

K

Kapp, B.S., Frysinger, R.C., Gallagher, M., and Haselton, J.R. (1979). Amygdala central nucleus lesions: effect on heart rate conditioning in the rabbit. Physiol. Behav. *23*, 1109–1117.

Karmarkar, U.R., and Buonomano, D.V. (2006). Different forms of homeostatic plasticity are engaged with distinct temporal profiles. Eur. J. Neurosci. *23*, 1575–1584.

Kemenes, I., Straub, V.A., Nikitin, E.S., Staras, K., O'Shea, M., Kemenes, G., and Benjamin, P.R. (2006). Role of Delayed Nonsynaptic Neuronal Plasticity in Long-Term Associative Memory. Curr. Biol. *16*, 1269–1279.

Kim, J.J., and Fanselow, M.S. (1992). Modality-specific retrograde amnesia of fear. Science *256*, 675–677.

Kim, J.J., and Jung, M.W. (2006). Neural circuits and mechanisms involved in Pavlovian fear conditioning: A critical review. Neurosci. Biobehav. Rev. *30*, 188–202.

Kim, J.J., Rison, R.A., and Fanselow, M.S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. Behav. Neurosci. *107*, 1093–1098.

Kim, J., Wei, D.S., and Hoffman, D.A. (2005). Kv4 potassium channel subunits control action potential repolarization and frequency-dependent broadening in rat hippocampal CA1 pyramidal neurones. J. Physiol. *569*, 41–57.

Kiss, T. (2008). Persistent Na-channels: origin and function. A review. Acta. Biol. Hung. *59 Suppl*, 1–12.

Knapska, E., Macias, M., Mikosz, M., Nowak, A., Owczarek, D., Wawrzyniak, M., Pieprzyk, M., Cymerman, I.A., Werka, T., Sheng, M., et al. (2012). Functional anatomy of neural circuits regulating fear and extinction. Proc. Natl. Acad. Sci. USA *109*, 17093–17098.

Knapska, E., and Maren, S. (2009). Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear. Learn. Memory *16*, 486–493.

Knapska, E., Radwanska, K., Werka, T., and Kaczmarek, L. (2007). Functional Internal Complexity of Amygdala: Focus on Gene Activity Mapping After Behavioral Training and Drugs of Abuse. Physiol. Rev. *87*, 1113–1173.

Kole, M.H.P., Letzkus, J.J., and Stuart, G.J. (2007). Axon initial segment Kv1 channels control axonal action potential waveform and synaptic efficacy. Neuron *55*, 633–647.

Kole, M.H.P., Hallermann, S., and Stuart, G.J. (2006). Single Ih channels in pyramidal neuron dendrites: properties, distribution, and impact on action potential output. J. Neurosci. *26*, 1677–1687.

Krettek, J.E., and Price, J.L. (1977). The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J. Comp. Neurol. *171*, 157–191.

Kuo, A.G., Lee, G., McKay, B.M., and Disterhoft, J.F. (2008). Enhanced neuronal excitability in rat CA1 pyramidal neurons following trace eyeblink conditioning acquisition is not due to alterations in I M. Neurobiol. Learn. Mem. *89*, 125–133.

L

Lai, H.C., and Jan, L.Y. (2006). The distribution and targeting of neuronal voltage-gated ion channels. Nat. Rev. Neurosci. *7*, 548–562.

Landeira-Fernandez, J., DeCola, J.P., Kim, J.J., and Fanselow, M.S. (2006). Immediate Shock Deficit in Fear Conditioning: Effects of Shock Manipulations. Behav. Neurosci. *120*, 873–879.

Larkum, M.E., Kaiser, K.M., and Sakmann, B. (1999). Calcium electrogenesis in distal apical dendrites of layer 5 pyramidal cells at a critical frequency of back-propagating action potentials. Proc. Natl. Acad. Sci. *96*, 14600–14604.

Larkum, M.E., Nevian, T., Sandler, M., Polsky, A., and Schiller, J. (2009). Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. Science *325*, 756–760.

Larkum, M.E., Waters, J., Sakmann, B., and Helmchen, F. (2007). Dendritic spikes in apical dendrites of neocortical layer 2/3 pyramidal neurons. J. Neurosci. *27*, 8999–9008.

Laurent, V., and Westbrook, R.F. (2009). Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction. Learn. Memory *16*, 520–529.

Lauzon, N.M., Bishop, S.F., and Laviolette, S.R. (2009). Dopamine D1 versus D4 Receptors Differentially Modulate the Encoding of Salient versus Nonsalient Emotional Information in the Medial Prefrontal Cortex. J. Neurosci. *29*, 4836–4845.

Laviolette, S.R. (2005). A Subpopulation of Neurons in the Medial Prefrontal Cortex Encodes Emotional Learning with Burst and Frequency Codes through a Dopamine D4 Receptor-Dependent Basolateral Amygdala Input. J. Neurosci. *25*, 6066–6075.

Laviolette, S.R., and Grace, A.A. (2006). Cannabinoids Potentiate Emotional Learning Plasticity in Neurons of the Medial Prefrontal Cortex through Basolateral Amygdala Inputs. J. Neurosci. *26*, 6458–6468.

Lavzin, M., Rapoport, S., Polsky, A., Garion, L., and Schiller, J. (2012). Nonlinear dendritic processing determines angular tuning of barrel cortex neurons in vivo. Nature *490*, 397–401.

LeDoux, J.E. (2000). Emotion circuits in the brain. Annu. Rev. Neurosci. *23*, 155–184.

LeDoux, J. (2003). The emotional brain, fear, and the amygdala. Cell. Mol. Neurobiol. *23*, 727–738.

LeDoux, J.E., Cicchetti, P., Xagoraris, A., and Romanski, L.M. (1990b). The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. J. Neurosci. *10*, 1062–1069.

LeDoux, J.E., Farb, C., and Ruggiero, D.A. (1990a). Topographic organization of neurons in the acoustic thalamus that project to the amygdala. J. Neurosci. *10*, 1043–1054.

LeDoux, J.E., Sakaguchi, A., Iwata, J., and Reis, D.J. (1986). Interruption of projections from the medial geniculate body to an archi-neostriatal field disrupts the classical conditioning of emotional responses to acoustic stimuli. Neuroscience *17*, 615–627.

Lee, I., and Solivan, F. (2008). The roles of the medial prefrontal cortex and hippocampus in a spatial paired-association task. Learn. Mem. *15*, 357–367.

Leon, W.C., Bruno, M.A., Allard, S., Nader, K., and Cuello, A.C. (2010). Engagement of the PFC in consolidation and recall of recent spatial memory. Learn. Memory *17*, 297–305.

Lesburgueres, E., Gobbo, O.L., Alaux-Cantin, S., Hambucken, A., Trifilieff, P., and Bontempi, B. (2011). Early Tagging of Cortical Networks Is Required for the Formation of Enduring Associative Memory. Science *331*, 924–928.

Lesting, J., Narayanan, R.T., Kluge, C., Sangha, S., Seidenbecher, T., and Pape, H.-C. (2011). Patterns of Coupled Theta Activity in Amygdala-Hippocampal-Prefrontal Cortical Circuits during Fear Extinction. PLoS ONE *6*, e21714.

Letzkus, J.J., Kampa, B.M., and Stuart, G.J. (2006). Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. J. Neurosci. *26*, 10420–10429.

Lewis, A.S., and Chetkovich, D.M. (2011). HCN channels in behavior and neurological disease: Too hyper or not active enough? Mol. Cell. Neurosci. *46*, 357–367.

Li, C.-Y., Lu, J.-T., Wu, C.-P., Duan, S.-M., and Poo, M.-M. (2004). Bidirectional modification of presynaptic neuronal excitability accompanying spike timing-dependent synaptic plasticity. Neuron *41*, 257–268.

Likhtik, E. (2005). Prefrontal Control of the Amygdala. J. Neurosci. *25*, 7429–7437.

Liu, X., Ramirez, S., Pang, P.T., Puryear, C.B., Govindarajan, A., Deisseroth, K., and Tonegawa, S. (2012). Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature *484*, 381–385.

Logothetis, N.K., Eschenko, O., Murayama, Y., Augath, M., Steudel, T., Evrard, H.C., Besserve, M., and Oeltermann, A. (2012). nature11618. Nature *491*, 547–553.

Luján, R. (2010). Organisation of potassium channels on the neuronal surface. J. Chem. Neuroanat. *40*, 1–20.

Luo, L., Callaway, E.M., and Svoboda, K. (2008). Genetic dissection of neural circuits. Neuron *57*, 634–660.

Lupinsky, D., Moquin, L., and Gratton, A. (2010). Interhemispheric Regulation of the Medial Prefrontal Cortical Glutamate Stress Response in Rats. J. Neurosci. *30*, 7624–7633.

M

Madisen, L., Mao, T., Koch, H., Zhuo, J.-M., Berenyi, A., Fujisawa, S., Hsu, Y.-W.A., Garcia, A.J., Gu, X., Zanella, S., et al. (2012). A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. Nat. Neurosci. *15*, 793–802.

Madison, D.V., and Nicoll, R.A. (1982). Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. Nature *299*, 636–638.

Magee, J. (1999). Dendritic Ih normalizes temporal summation in hippocampal CA1 neurons. Nature *2*, 848.

Magee, J.C. (2000). Dendritic integration of excitatory synaptic input. Nat. Rev. Neurosci *1*, 181–190.

Magee, J.C. (2008). Dendritic voltage-gated ion channels. In Dendrites, J.G. Stuart, N. Spruston and M. Häusser, eds. (New York: Oxford University Press), pp. 225-250.

Magee, J.C., and Johnston, D. (1997). A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. Science *275*, 209–213.

Magee, J.C., and Johnston, D. (2005). Plasticity of dendritic function. Curr. Opin. Neurobiol. *15*, 334–342.

Major, G., Larkum, M.E., and Schiller, J. (2013). Active Properties of Neocortical Pyramidal Neuron Dendrites. Annu. Rev. Neurosci. *36*, 1–24.

Malenka, R.C., and Bear, M.F. (2004). LTP and LTD: an embarrassment of riches. Neuron *44*, 5–21.

Malin, S.A., and Nerbonne, J.M. (2002). Delayed rectifier K+ currents, IK, are encoded by Kv2 alpha-subunits and regulate tonic firing in mammalian sympathetic neurons. J. Neurosci. *22*, 10094–10105.

Mamiya, N., Fukushima, H., Suzuki, A., Matsuyama, Z., Homma, S., Frankland, P.W., and Kida, S. (2009). Brain Region-Specific Gene Expression Activation Required for Reconsolidation and Extinction of Contextual Fear Memory. J. Neurosci. *29*, 402–413.

Marek, R., Strobel, C., Bredy, T.W., and Sah, P. (2013). The amygdala and medial prefrontal cortex: partners in the fear circuit. J. Physiol. *591*, 2381–2391.

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., and Wu, C. (2004). Interneurons of the neocortical inhibitory system. Nat. Rev. Neurosci *5*, 793–807.

Maren, S. (2003). The amygdala, synaptic plasticity, and fear memory. Ann. N. Y. Acad. Sci. *985*, 106–113.

Maren, S., Aharonov, G., and Fanselow, M.S. (1996a). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. Behav. Neurosci. *110*, 718–726.

Maren, S., Aharonov, G., and Fanselow, M.S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behav. Brain Res. *88*, 261–274.

Maren, S., Aharonov, G., Stote, D.L., and Fanselow, M.S. (1996b). N-methyl-D-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. Behav. Neurosci. *110*, 1365–1374.

Maren, S., and Fanselow, M.S. (1995). Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation in vivo. J. Neurosci. *15*, 7548–7564.

Maren, S., Phan, K.L., and Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. Nat. Rev. Neurosci. *14*, 417–428.

Maren, S., Poremba, A., and Gabriel, M. (1991). Basolateral amygdaloid multi-unit neuronal correlates of discriminative avoidance learning in rabbits. Brain Research *549*, 311–316.

Maren, S., and Quirk, G.J. (2004). Neuronal signalling of fear memory. Nat. Rev. Neurosci. *5*, 844–852.

Matthews, E.A., Weible, A.P., Shah, S., and Disterhoft, J.F. (2008). The BK-mediated fAHP is modulated by learning a hippocampus-dependent task. Proc. Natl. Acad. Sci. USA *105*, 15154–15159.

Maviel, T., Durkin, T.P., Menzaghi, F., and Bontempi, B. (2004). Sites of neocortical reorganization critical for remote spatial memory. Science *305*, 96–99.

McClelland, J.L., McNaughton, B.L., and O'Reilly, R.C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the

successes and failures of connectionist models of learning and memory. Psychol. Rev. *102*, 419–457.

McDonald, A.J. (1991). Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. Neuroscience *44*, 1–14.

McDonald, A.J. (1998). Cortical pathways to the mammalian amygdala. Prog. Neurobiol. *55*, 257–332.

McDonald, A.J., Mascagni, F., and Guo, L. (1996). Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. Neuroscience *71*, 55–75.

McGaugh, J.L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu. Rev. Neurosci. *27*, 1–28.

McKay, B.M., Matthews, E.A., Oliveira, F.A., and Disterhoft, J.F. (2009). Intrinsic Neuronal Excitability Is Reversibly Altered by a Single Experience in Fear Conditioning. J. Neurphysiol. *102*, 2763–2770.

McKay, B.M., Oh, M.M., Galvez, R., Burgdorf, J., Kroes, R.A., Weiss, C., Adelman, J.P., Moskal, J.R., and Disterhoft, J.F. (2012). Increasing SK2 channel activity impairs associative learning. J. Neurophysiol. *108*, 863–870.

McKinney, B.C., and Murphy, G.G. (2006). The L-Type voltage-gated calcium channel Cav1. 3 mediates consolidation, but not extinction, of contextually conditioned fear in mice. Learn. Memory *13*, 584–589.

Messier, C., Mourre, C., Bontempi, B., Sif, J., Lazdunski, M., and Destrade, C. (1991). Effect of apamin, a toxin that inhibits Ca(2+)-dependent K+ channels, on learning and memory processes. Brain Res. *551*, 322–326.

Migliore, M., and Shepherd, G.M. (2002). Emerging rules for the distributions of active dendritic conductances. Nat. Rev. Neurosci. *3*, 362–370.

Milad, M.R., Vidal-Gonzalez, I., and Quirk, G.J. (2004). Electrical Stimulation of Medial Prefrontal Cortex Reduces Conditioned Fear in a Temporally Specific Manner. Behav. Neurosci. *118*, 389–394.

Miller, C. (2000). An overview of the potassium channel family. Genome Biol. *1*, 1-5.

Miller, E.K., and Cohen, J.D. (2001). An integrative theory of prefrontal cortex function. Annu. Rev. Neurosci. 24, 167–202.

Morgan, M.A., and LeDoux, J.E. (1995). Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. Behavioral Neuroscience *109*, 681–688.

Morgan, M.A., Romanski, L.M., and LeDoux, J.E. (1993). Extinction of emotional learning: contribution of medial prefrontal cortex. Neurosci. Lett. *163*, 109–113.

Morgan, M.A., Schulkin, J., and LeDoux, J.E. (2003). Ventral medial prefrontal cortex and emotional perseveration: the memory for prior extinction training. Behav. Brain Res. *146*, 121–130.

Morris, R.G., and Frey, U. (1997). Hippocampal synaptic plasticity: role in spatial learning or the automatic recording of attended experience? Philos. Trans. R. Soc. London [Biol.] *352*, 1489–1503.

Morrow, B.A., Elsworth, J.D., Inglis, F.M., and Roth, R.H. (1999). An antisense oligonucleotide reverses the footshock-induced expression of fos in the rat medial prefrontal cortex and the subsequent expression of conditioned fear-induced immobility. J. Neurosci. *19*, 5666–5673.

Motanis, H., Maroun, M., and Barkai, E. (2014). Learning-induced bidirectional plasticity of intrinsic neuronal excitability reflects the valence of the outcome. Cereb. Cortex *24*, 1075–1087. First published December 12, 2012, doi: 10.1093/cercor/bhs394

Moyer, J.R., Thompson, L.T., and Disterhoft, J.F. (1996). Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. J. Neurosci. *16*, 5536–5546.

Mozzachiodi, R., and Byrne, J.H. (2010). More than synaptic plasticity: role of nonsynaptic plasticity in learning and memory. Trends Neurosci. *33*, 17–26.

Mueller, D., and Cahill, S.P. (2010). Noradrenergic modulation of extinction learning and exposure therapy. Behav. Brain Res. *208*, 1–11.

Mueller, D., Porter, J.T., and Quirk, G.J. (2008). Noradrenergic Signaling in Infralimbic Cortex Increases Cell Excitability and Strengthens Memory for Fear Extinction. J. Neurosci. *28*, 369–375.

Muigg, P., Hetzenauer, A., Hauer, G., Hauschild, M., Gaburro, S., Frank, E., Landgraf, R., and Singewald, N. (2008). Impaired extinction of learned fear in rats selectively bred for high anxiety--evidence of altered neuronal processing in prefrontal-amygdala pathways. Eur. J. Neurosci. *28*, 2299–2309.

N

Nadel, L., Samsonovich, A., Ryan, L. and Moscovitch, M. (2000). Multiple trace theory of human memory: computational, neuroimaging, and neuropsychological results. Hippocampus *10*, 352–68.

Narayanan, R., and Johnston, D. (2007). Long-term potentiation in rat hippocampal neurons is accompanied by spatially widespread changes in intrinsic oscillatory dynamics and excitability. Neuron *56*, 1061–1075.

Narayanan, R.T., Seidenbecher, T., Kluge, C., Bergado, J., Stork, O., and Pape, H.-C. (2007).

Dissociated theta phase synchronization in amygdalo- hippocampal circuits during various stages of fear memory. Eur. J. Neurosci. *25*, 1823–1831.

Nolan, M.F., Malleret, G., Dudman, J.T., Buhl, D.L., Santoro, B., Gibbs, E., Vronskaya, S., Buzsáki, G., Siegelbaum, S.A., Kandel, E.R., et al. (2004). A Behavioral Role for Dendritic Integration. Cell *119*, 719–732.

Norris, A.J., and Nerbonne, J.M. (2010). Molecular Dissection of IA in Cortical Pyramidal Neurons Reveals Three Distinct Components Encoded by Kv4.2, Kv4.3, and Kv1.4 - Subunits. J. Neurosci. *30*, 5092–5101.

Notomi, T., and Shigemoto, R. (2004). Immunohistochemical localization of Ih channel subunits, HCN1-4, in the rat brain. J. Comp. Neurol. *471*, 241–276.

Nusser, Z. (2012). Differential subcellular distribution of ion channels and the diversity of neuronal function. Curr. Opin. Neurobiol. *22*, 366–371.

O

Oberlaender, M., Boudewijns, Z.S., Kleele, T., Mansvelder, H.D., Sakmann, B. and de Kock, C.P. (2011). Three-dimensional axon morphologies of individual layer 5 neurons indicate cell type-specific intracortical pathways for whisker motion and touch. Proc. Natl. Acad. Sci. USA *108,* 4188-93.

Oh, M.M., Kuo, A.G., Wu, W.W., Sametsky, E.A., and Disterhoft, J.F. (2003). Watermaze learning enhances excitability of CA1 pyramidal neurons. J. Neurophysiol. *90*, 2171–2179.

Oh, M.M., McKay, B.M., Power, J.M., and Disterhoft, J.F. (2009). Learning-related postburst afterhyperpolarization reduction in CA1 pyramidal neurons is mediated by protein kinase A. Proc. Natl. Acad. Sci. USA *106*, 1620–1625.

O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Research *34*, 171–175.

O'Keefe, J., and Nadel, L. (1978). The hippocampus as a cognitive map (Oxford: Clarendon Press).

O'Leary, T., van Rossum, M.C.W., and Wyllie, D.J.A. (2010). Homeostasis of intrinsic excitability in hippocampal neurones: dynamics and mechanism of the response to chronic depolarization. J. Physiol. *588*, 157–170.

Öngür, D., and Price, J.L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cereb. Cortex *10*, 206–219.

Orsini, C.A., Kim, J.H., Knapska, E., and Maren, S. (2011). Hippocampal and Prefrontal Projections to the Basal Amygdala Mediate Contextual Regulation of Fear after Extinction. J. Neurosci. *31*, 17269–17277.

Orsini, C.A., and Maren, S. (2012). Neural and cellular mechanisms of fear and extinction memory formation. Neurosci. Biobehav. Rev. *36*, 1773–1802.

Oswald, B.B., Maddox, S.A., and Powell, D.A. (2008). Prefrontal control of trace eyeblink conditioning in rabbits: role in retrieval of the CR? Behav. Neurosci. *122*, 841–848.

P

Packer, A.M., Roska, B., and Häusser, M. (2013). Targeting neurons and photons for optogenetics. Nat. Neurosci. *16*, 805–815.

Pape, H.C. (1996). Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. Annu. Rev. Physiol. *58*, 299–327.

Pape, H.C., and Paré, D. (2010). Plastic Synaptic Networks of the Amygdala for the Acquisition, Expression, and Extinction of Conditioned Fear. Physiol. Rev. *90*, 419–463.

Paré, D. (2002). Mechanisms of Pavlovian fear conditioning: has the engram been located? Trends Neurosci. *25*, 436–438.

Paré, D. (2004). New Vistas on Amygdala Networks in Conditioned Fear. J. Neurophysiol. *92*, 1–9.

Park, K.-S., Yang, J.-W., Seikel, E., and Trimmer, J.S. (2008). Potassium channel phosphorylation in excitable cells: providing dynamic functional variability to a diverse family of ion channels. Physiology (Bethesda) *23*, 49–57.

Pascoe, J.P., and Kapp, B.S. (1985). Electrophysiological characteristics of amygdaloid central nucleus neurons during Pavlovian fear conditioning in the rabbit. Behavioural Brain Res. *16*, 117–133.

Pavlov, I.P. (1927). Conditioned Reflexes: An Investigation of the Physiological Activity of the Cereb. Cortex. (London: Oxford University Press)

Paxinos, G. and Franklin, K.B.J. (2001). The Mouse Brain in Stereotaxic Coordinates. (London: Academic Press).

Paxinos, G. and Watson, C. (1997). The Rat Brain in Stereotaxic Coordinates (San Diego, CA: Academic Press).

Paz, R., Bauer, E.P., and Pare, D. (2008). Theta synchronizes the activity of medial prefrontal neurons during learning. Learn. Memory *15*, 524–531.

Paz, R., Bauer, E.P., and Paré, D. (2009). Measuring correlations and interactions among four simultaneously recorded brain regions during learning. J. Neurophysiol. *101*, 2507–2515.

Peters, H.C., Hu, H., Pongs, O., Storm, J.F., and Isbrandt, D. (2004). Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. Nat. Neurosci. *8*, 51–60.

Peters, J., Kalivas, P.W., and Quirk, G.J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn. Memory *16*, 279–288.

Peyrache, A., Khamassi, M., Benchenane, K., Wiener, S.I., and Battaglia, F.P. (2009). Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. Nature *12*, 919–926.

Pfeiffer, U.J., and Fendt, M. (2006). Prefrontal dopamine D4 receptors are involved in encoding fear extinction. Neuroreport *17*, 847–850.

Phelps, E.A., and LeDoux, J.E. (2005). Contributions of the Amygdala to Emotion Processing: From Animal Models to Human Behavior. Neuron *48*, 175–187.

Phillips, R.G., and LeDoux, J.E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav. Neurosci. *106*, 274–285.

Pitkänen, A., Jolkkonen, E., and Kemppainen, S. (2000a). Anatomic heterogeneity of the rat amygdaloid complex. Folia Morphol. (Warsz) *59*, 1–23.

Pitkänen, A., Pikkarainen, M., Nurminen, N., and Ylinen, A. (2000b). Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Ann. N. Y. Acad. Sci. *911*, 369–391.

Pitkänen, A., Savander, V., and LeDoux, J.E. (1997). Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci. *20*, 517–523.

de Polavieja, G.G. (2005). Stimulus History Reliably Shapes Action Potential Waveforms of Cortical Neurons. J. Neurosci. *25*, 5657–5665.

Poolos, N.P., Migliore, M., and Johnston, D. (2002). Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. Nat. Neurosci. *5*, 767–774.

Poorthuis, R.B., Bloem, B., Schak, B., Wester, J., de Kock, C.P.J., and Mansvelder, H.D. (2013). Layer-specific modulation of the prefrontal cortex by nicotinic acetylcholine receptors. Cereb. Cortex *23*, 148–161.

Popa, D., Duvarci, S., Popescu, A.T., Lena, C., and Pare, D. (2010). Coherent amygdalocortical theta promotes fear memory consolidation during paradoxical sleep. Proc. Natl. Acad. Sci. USA *107*, 6516–6519.

Pratt, K.G., and Aizenman, C.D. (2007). Homeostatic regulation of intrinsic excitability and synaptic transmission in a developing visual circuit. J. Neurosci. *27*, 8268–8277.

Q

Quinn, J.J., Ma, Q.D., Tinsley, M.R., Koch, C., and Fanselow, M.S. (2008). Inverse temporal contributions of the dorsal hippocampus and medial prefrontal cortex to the expression of long-term fear memories. Learn. Memory *15*, 368–372.

Quirk, G.J., and Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology *33*, 56–72.

Quirk, G.J., Likhtik, E., Pelletier, J.G., and Paré, D. (2003). Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. J. Neurosci. *23*, 8800–8807.

Quirk, G.J., Repa, C., and LeDoux, J.E. (1995). Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. Neuron *15*, 1029–1039.

R

Ray, J.P., and Price, J.L. (1992). The organization of the thalamocortical connections of the mediodorsal thalamic nucleus in the rat, related to the ventral forebrain-prefrontal cortex topography. J. Comp. Neurol. *323*, 167–197.

Reijmers, L.G., Perkins, B.L., Matsuo, N., and Mayford, M. (2007). Localization of a Stable Neural Correlate of Associative Memory. Science *317*, 1230–1233.

Remy, S., Csicsvari, J., and Beck, H. (2009). Activity-dependent control of neuronal output by local and global dendritic spike attenuation. Neuron *61*, 906–916.

Restivo, L., Vetere, G., Bontempi, B., and Ammassari-Teule, M. (2009). The Formation of Recent and Remote Memory Is Associated with Time-Dependent Formation of Dendritic Spines in the Hippocampus and Anterior Cingulate Cortex. J. Neurosci. *29*, 8206–8214.

Ridderinkhof, K.R., Ullsperger, M., Crone, E.A., and Nieuwenhuis, S. (2004). The role of the medial frontal cortex in cognitive control. Science 306, 443–447.

Robinson, R.B., and Siegelbaum, S.A. (2003). Hyperpolarization-activated cation currents: from molecules to physiological function. Annu. Rev. Physiol. *65*, 453–480.

Rodrigues, S.M., Schafe, G.E., and LeDoux, J.E. (2001). Intra-amygdala blockade of the NR2B subunit of the NMDA receptor disrupts the acquisition but not the expression of fear conditioning. J. Neurosci. *21*, 6889–6896.

Rogawski, M.A. (2000). KCNQ2/KCNQ3 K+ channels and the molecular pathogenesis of epilepsy: implications for therapy. Trends Neurosci. *23*, 393–398.

Romanski, L.M., Clugnet, M.C., Bordi, F., and LeDoux, J.E. (1993). Somatosensory and auditory convergence in the lateral nucleus of the amygdala. Behav. Neurosci. *107*, 444–450.

Romanski, L.M., and LeDoux, J.E. (1993). Information cascade from primary auditory cortex to the amygdala: corticocortical and corticoamygdaloid projections of temporal cortex in the rat. Cereb. Cortex *3*, 515–532.

Rose, J.E. and Woolsey, C.N. (1948). The orbitofrontal cortex and its connections with the mediodorsal nucleus in rabbit, sheep and cat. Res. Publ. Assn. Nerv. Dis. *27*, 210–232.

Rosenkranz, J.A. (2011). Neuronal Activity Causes Rapid Changes of Lateral Amygdala Neuronal Membrane Properties and Reduction of Synaptic Integration and Synaptic Plasticity In Vivo. J. Neurosci. *31*, 6108–6120.

Rosenkranz, J.A., Frick, A., and Johnston, D. (2009). Kinase-dependent modification of dendritic excitability after long-term potentiation. J. Physiol. *587*, 115–125.

Rudy, J.W., Huff, N.C., and Matus-Amat, P. (2004). Understanding contextual fear conditioning: insights from a two-process model. Neurosci. Biobehav. Rev. *28*, 675–685.

Rudy, J.W., Barrientos, R.M., and O'Reilly, R.C. (2002). Hippocampal formation supports conditioning to memory of a context. Behav. Neurosci.*116*, 530–538.

Rudy, J.W., and O'Reilly, R.C. (2001). Conjunctive representations, the hippocampus, and contextual fear conditioning. Cognitive, Affective, & Behavioral Neuroscience *1*, 66–82.

Runyan, J.D. (2004). A Role for Prefrontal Cortex in Memory Storage for Trace Fear Conditioning. J. Neurosci. *24*, 1288–1295.

Rushworth, M.F.S., Noonan, M.P., Boorman, E.D., Walton, M.E., and Behrens, T.E. (2011). Frontal Cortex and Reward-Guided Learning and Decision-Making. Neuron *70*, 1054–1069.

S

Saar, D., Grossman, Y., and Barkai, E. (1998). Reduced after-hyperpolarization in rat piriform cortex pyramidal neurons is associated with increased learning capability during operant conditioning. Eur. J. Neurosci. *10*, 1518–1523.

Sacchetti, B., Baldi, E., Lorenzini, C.A., and Bucherelli, C. (2003). Role of the neocortex in consolidation of fear conditioning memories in rats. Exp. Brain Res. *152*, 323–328.

Sah, P., and Faber, E.S.L. (2002). Channels underlying neuronal calcium-activated potassium currents. Prog. Neurobiol. *66*, 345–353.

Sah, P., Faber, E.S.L., Lopez De Armentia, M., and Power, J. (2003). The amygdaloid complex: anatomy and physiology. Physiol. Rev. *83*, 803–834.

Sah, P., and Westbrook, R.F. (2008). Behavioural neuroscience: The circuit of fear. Nature *454*, 589–590.

Sailer, C.A., Kaufmann, W.A., Marksteiner, J., and Knaus, H.-G. (2004). Comparative immunohistochemical distribution of three small-conductance Ca2+-activated potassium channel subunits, SK1, SK2, and SK3 in mouse brain. Mol. Cell. Neurosci. *26*, 458–469.

Sanders, M.J., Wiltgen, B.J., and Fanselow, M.S. (2003). The place of the hippocampus in fear conditioning. Eur. J. Pharmacol. *463*, 217–223.

Santini, E., Ge, H., Ren, K., Peña de Ortiz, S., and Quirk, G.J. (2004). Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. J. Neurosci. *24*, 5704–5710.

Santini, E., and Porter, J.T. (2010). M-type potassium channels modulate the intrinsic excitability of infralimbic neurons and regulate fear expression and extinction. J. Neurosci. *30*, 12379–12386.

Santini, E., Quirk, G.J., and Porter, J.T. (2008). Fear Conditioning and Extinction Differentially Modify the Intrinsic Excitability of Infralimbic Neurons. J. Neurosci. *28*, 4028–4036.

Santini, E., Sepulveda-Orengo, M., and Porter, J.T. (2012). npp201252a. Neuropsychopharmacology *37*, 2047–2056.

Sausbier, U., Sausbier, M., Sailer, C.A., Arntz, C., Knaus, H.-G., Neuhuber, W., and Ruth, P. (2006). Ca2+ -activated K+ channels of the BK-type in the mouse brain. Histochem. Cell Biol. *125*, 725–741.

Schacter, D.L. and Tulving, E. (1994). Memory Systems (Cambridge, MA: MIT Press).

Schaefer, A.T. (2003). Coincidence Detection in Pyramidal Neurons Is Tuned by Their Dendritic Branching Pattern. J. Neurophysiol. *89*, 3143–3154.

Schrader, L.A., Anderson, A.E., Mayne, A., Pfaffinger, P.J., and Sweatt, J.D. (2002). PKA modulation of Kv4.2-encoded A-type potassium channels requires formation of a supramolecular complex. J. Neurosci. *22*, 10123–10133.

Schreurs, B.G., Gusev, P.A., Tomsic, D., Alkon, D.L., and Shi, T. (1998). Intracellular correlates of acquisition and long-term memory of classical conditioning in Purkinje cell dendrites in slices of rabbit cerebellar lobule HVI. J. Neurosci. *18*, 5498–5507.

Schreurs, B.G., Tomsic, D., Gusev, P.A., and Alkon, D.L. (1997). Dendritic excitability microzones and occluded long-term depression after classical conditioning of the rabbit's nictitating membrane response. J. Neurophysiol. *77*, 86–92.

Schubert, D., Kötter, R., and Staiger, J.F. (2007). Mapping functional connectivity in barrel-related columns reveals layer- and cell type-specific microcircuits. Brain. Struct. Funct. *212*, 107–119.

Sehgal, M., Ehlers, V.L., and Moyer, J.R. (2014). Learning enhances intrinsic excitability in a subset of lateral amygdala neurons. Learn. Memory *21*, 161–170.

Senn, V., Wolff, S.B.E., Herry, C., Grenier, F., Ehrlich, I., Gründemann, J., Fadok, J.P., Müller, C., Letzkus, J.J., and Lüthi, A. (2014). Long-range connectivity defines behavioral specificity of amygdala neurons. Neuron *81*, 428–437.

Shackman, A.J., Salomons, T.V., Slagter, H.A., Fox, A.S., Winter, J.J., and Da- vidson, R.J. (2011). The integration of negative affect, pain and cognitive control in the cingulate cortex. Nat. Rev. Neurosci. 12, 154–167.

Shepherd, G.M.G. (2009). Intracortical cartography in an agranular area. Front. Neurosci. *3*, 337–343.

Shinnick-Gallagher, P., McKernan, M.G., Xie, J., and Zinebi, F. (2003). L-type voltage-gated calcium channels are involved in the in vivo and in vitro expression of fear conditioning. Ann. N. Y. Acad. Sci. *985*, 135–149.

Shu, Y., Hasenstaub, A., Duque, A., Yu, Y., and McCormick, D.A. (2006). Modulation of intracortical synaptic potentials by presynaptic somatic membrane potential. Nature *441*, 761–765.

Shu, Y., Yu, Y., Yang, J., and McCormick, D.A. (2007). Selective control of cortical axonal spikes by a slowly inactivating K+ current. Proc. Natl. Acad. Sci. USA *104*, 11453–11458.

Siapas, A.G., Lubenov, E.V., and Wilson, M.A. (2005). Prefrontal Phase Locking to Hippocampal Theta Oscillations. Neuron *46*, 141–151.

Sierra-Mercado, D., Padilla-Coreano, N., and Quirk, G.J. (2011). Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology *36*, 529–538.

Sigurdsson, T., Doyère, V., Cain, C.K., and LeDoux, J.E. (2007). Long-term potentiation in the amygdala: A cellular mechanism of fear learning and memory. Neuropharmacology *52*, 215–227.

Sirota, A., Montgomery, S., Fujisawa, S., Isomura, Y., Zugaro, M., and Buzsáki, G. (2008). Entrainment of Neocortical Neurons and Gamma Oscillations by the Hippocampal Theta Rhythm. Neuron *60*, 683–697.

Sjöström, P.J., and Häusser, M. (2006). A cooperative switch determines the sign of synaptic plasticity in distal dendrites of neocortical pyramidal neurons. Neuron *51*, 227–238.

Sjöström, P.J., Rancz, E.A., Roth, A., and Häusser, M. (2008). Dendritic excitability and synaptic plasticity. Physiol. Rev. *88*, 769–840.

Smith, G.B., Heynen, A.J., and Bear, M.F. (2009). Bidirectional synaptic mechanisms of ocular dominance plasticity in visual cortex. Phil. Tr. R. Soc. B: [Biol] *364*, 357–367.

Song, C., Detert, J.A., Sehgal, M., and Moyer, J.R. (2012). Trace fear conditioning enhances synaptic and intrinsic plasticity in rat hippocampus. J. Neurophysiol.*107*, 3397–3408.

Song, C., Ehlers, VL., Aitken, JC., Bula, T., Moyer Jr, JR (2013, November). Effect of trace fear conditioning and extinction on mPFC-amygdala projection neurons. Poster session presented at the meeting of the Society for Neuroscience, San Diego, CA.

Sotres-Bayon, F., Diaz-Mataix, L., Bush, D.E.A., and LeDoux, J.E. (2009). Dissociable Roles for the Ventromedial Prefrontal Cortex and Amygdala in Fear Extinction: NR2B Contribution. Cereb. Cortex *19*, 474–482.

Sotres-Bayon, F., and Quirk, G.J. (2010). Prefrontal control of fear: more than just extinction. Curr. Opin. Neurobiol. *20*, 231–235.

Sotres-Bayon, F., Sierra-Mercado, D., Pardilla-Delgado, E., and Quirk, G.J. (2012). Gating of Fear in Prelimbic Cortex by Hippocampal and Amygdala Inputs. Neuron *76*, 804–812.

Spruston, N. (2008). Pyramidal neurons: dendritic structure and synaptic integration. Nat. Rev. Neurosci *9*, 206–221.

Spruston, N., Schiller, Y., Stuart, G., and Sakmann, B. (1995). Activity-dependent action potential invasion and calcium influx into hippocampal CA1 dendrites. Science *268*, 297–300.

Squire, L.R. (1992). Declarative and nondeclarative memory: multiple brain systems supporting learning and memory. J. Cogn. Neurosci. *4*, 232–243.

Squire, L.R. (2004). Memory systems of the brain: A brief history and current perspective. Neurobiol. Learn. Mem. *82*, 171–177.

Squire, L.R., and Bayley, P.J. (2007). The neuroscience of remote memory. Curr. Opin. Neurobiol. *17*, 185–196.

van der Staay, F.J., Fanelli, R.J., Blokland, A., and Schmidt, B.H. (1999). Behavioral effects of apamin, a selective inhibitor of the SK(Ca)-channel, in mice and rats. Neurosci. Biobehav. Rev. *23*, 1087–1110.

Stackman, R.W., Hammond, R.S., Linardatos, E., Gerlach, A., Maylie, J., Adelman, J.P., and Tzounopoulos, T. (2002). Small conductance Ca2+-activated K+ channels modulate synaptic plasticity and memory encoding. J. Neurosci. *22*, 10163–10171.

Stackman, R.W., Bond, C.T., and Adelman, J.P. (2008). Contextual memory deficits observed in mice overexpressing small conductance Ca2+-activated K+ type 2 (KCa2.2, SK2) channels are caused by an encoding deficit. Learn. Memory *15*, 208–213.

Staiger, J.F., Flagmeyer, I., Schubert, D., Zilles, K., Kötter, R., and Luhmann, H.J. (2004). Functional diversity of layer IV spiny neurons in rat somatosensory cortex: quantitative morphology of electrophysiologically characterized and biocytin labeled cells. Cereb. Cortex *14*, 690–701.

Stevenson, C.W. (2011). Role of amygdala-prefrontal cortex circuitry in regulating the expression of contextual fear memory. Neurobiol. Learn. Memory *96*, 315–323.

Stocker, M., Hirzel, K., D'hoedt, D., and Pedarzani, P. (2004). Matching molecules to function: neuronal Ca2+-activated K+ channels and afterhyperpolarizations. Toxicon *43*, 933–949.

Streb, J.M., and Smith, K. (1955). Frontal lobotomy and the elimination of conditioned anxiety in the rat. J. Comp. Physiol. Psychol. *48*, 126–129.

Stuart, G.J., and Hausser, M. (2001). Dendritic coincidence detection of EPSPs and action potentials. Nat. Neurosci. *4*, 63–71.

Stuart, G.J., and Sakmann, B. (1994). Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. Nature *367*, 69–72.

Stuart, G., Schiller, J., and Sakmann, B. (1997a). Action potential initiation and propagation in rat neocortical pyramidal neurons. J. Physiol. *505 (Pt 3)*, 617–632.

Stuart, G., Spruston, N., Sakmann, B., and Hausser, M. (1997b). Action potential initiation and backpropagation in neurons of the mammalian CNS. Trends Neurosci. *20*, 125–131.

Stevenson, C.W. (2011). Role of amygdala-prefrontal cortex circuitry in regulating the expression of contextual fear memory. Neurobiology of Learn. Memory *96*, 315–323.

van Strien, N.M., Cappaert, N.L.M., and Witter, M.P. (2009). The anatomy of memory: an interactive overview of the parahippocampal–hippocampal network. Nat. Rev. Neurosci. *10*, 272–282.

Szlapczynska, M**.**, Bonnan, A., Ginger, M, and Frick, A. (2014). Plasticity and pathology of dendritic intrinsic excitability. In Horizons in Neuroscience Research, Vol 14, A. Costa and E. Villalba, eds. (New York: Nova Science Publishers), pp. 41-88.

T

Takehara, K., Kawahara, S., and Kirino, Y. (2003). Time-dependent reorganization of the brain components underlying memory retention in trace eyeblink conditioning. J. Neurosci. *23*, 9897–9905.

Tang, J., Ko, S., Ding, H.-K., Qiu, C.-S., Calejesan, A.A., and Zhuo, M. (2005). Pavlovian fear memory induced by activation in the anterior cingulate cortex. Mol. Pain *1*, 6.

Taube, J.S., and Schwartzkroin, P.A. (1988). Mechanisms of long-term potentiation: a current-source density analysis. J. Neurosci. *8*, 1645–1655.

Tayler, K.K., Tanaka, K.Z., Reijmers, L.G., and Wiltgen, B.J. (2013). Reactivation of Neural Ensembles during the Retrieval of Recent and Remote Memory. Curr. Biol. *23*, 99–106.

Teixeira, C.M., Pomedli, S.R., Maei, H.R., Kee, N., and Frankland, P.W. (2006). Involvement of the anterior cingulate cortex in the expression of remote spatial memory. J. Neurosci. *26*, 7555–7564.

Thierry, A.M., Gioanni, Y., Dégenètais, E., and Glowinski, J. (2000). Hippocampo-prefrontal cortex pathway: anatomical and electrophysiological characteristics. Hippocampus *10*, 411–419.

Thomas, K.L., Hall, J., and Everitt, B.J. (2002). Cellular imaging with zif268 expression in the rat nucleus accumbens and frontal cortex further dissociates the neural pathways activated following the retrieval of contextual and cued fear memory. Eur. J. Neurosci. *16*, 1789–1796.

Thompson, L.T., Moyer, J.R., and Disterhoft, J.F. (1996). Transient changes in excitability of rabbit CA3 neurons with a time course appropriate to support memory consolidation. J. Neurophysiol. *76*, 1836–1849.

Thomson, A.M., and Lamy, C. (2007). Functional maps of neocortical local circuitry. Front. Neurosci. *1*, 19–42.

Thompson, R.F., Swain, R., Clark, R., and Shinkman, P. (2000). Intracerebellar conditioning--Brogden and Gantt revisited. Behav. Br. Res. *110*, 3–11.

Trimmer, J.S., and Rhodes, K.J. (2004). Localization of voltage-gated ion channels in mammalian brain. Annu. Rev. Physiol. *66*, 477–519.

Tronel, S., Feenstra, M.G.P., and Sara, S.J. (2004). Noradrenergic action in prefrontal cortex in the late stage of memory consolidation. Learn. Memory *11*, 453–458.

Tronel, S., and Sara, S.J. (2003). Blockade of NMDA receptors in prelimbic cortex induces an enduring amnesia for odor-reward associative learning. J. Neurosci. *23*, 5472–5476.

Tronson, N.C., Corcoran, K.A., Jovasevic, V., and Radulovic, J. (2012). Fear conditioning and extinction: emotional states encoded by distinct signaling pathways. Trends Neurosci. *35*, 145–155.

Tulogdi, Á., Sörös, P., Tóth, M., Nagy, R., Biró, L., Aliczki, M., Klausz, B., Mikics, É., and Haller, J. (2012). Temporal changes in c-Fos activation patterns induced by conditioned fear. Brain Res. Bull. *88*, 359–370.

Turrigiano, G., Abbott, L.F., and Marder, E. (1994). Activity-dependent changes in the intrinsic properties of cultured neurons. Science *264*, 974–977.

Turrigiano, G., LeMasson, G., and Marder, E. (1995). Selective regulation of current densities underlies spontaneous changes in the activity of cultured neurons. J. Neurosci. *15*, 3640–3652.

U

Ulrich, D. (2002). Dendritic resonance in rat neocortical pyramidal cells. J. Neurophysiol. *87*, 2753–2759.

Uylings, H.B., and van Eden, C.G. (1990). Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans. Prog. Brain Res. *85*, 31–62.

V

Vacher, H., Mohapatra, D.P., and Trimmer, J.S. (2008). Localization and targeting of voltage-dependent ion channels in mammalian central neurons. Physiol. Rev. *88*, 1407–1447.

Varga, A.W. (2004). Calcium-Calmodulin-Dependent Kinase II Modulates Kv4.2 Channel Expression and Upregulates Neuronal A-Type Potassium Currents. Journal of Neuroscience *24*, 3643–3654.

Vazdarjanova, A., and McGaugh, J.L. (1998). Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. Proc. Natl. Acad. Sci. USA *95*, 15003–15007.

Vetere, G., Restivo, L., Cole, C.J., Ross, P.J., Ammassari-Teule, M., Josselyn, S.A., and Frankland, P.W. (2011). Spine growth in the anterior cingulate cortex isnecessary for the consolidation of contextual fearmemory. Proc. Natl. Acad. Sci. USA. *108*, 8456–8460.

Vertes, R.P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse *51*, 32–58.

Vertes, R.P. (2006). Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience *142*, 1–20.

Vertes, R.P., Hoover, W.B., Szigeti-Buck, K., and Leranth, C. (2007). Nucleus reuniens of the midline thalamus: Link between the medial prefrontal cortex and the hippocampus. Brain Res. Bull. *71*, 601–609.

Vetter, P., Roth, A., and Hausser, M. (2001). Propagation of action potentials in dendrites depends on dendritic morphology. J. Neurophysiol. *85*, 926–937.

Vick, K.A., IV, Guidi, M., and Stackman, R.W., Jr (2010). In vivo pharmacological manipulation of small conductance Ca2+-activated K+ channels influences motor behavior, object memory and fear conditioning. Neuropharmacology *58*, 650–659.

Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S.L., and Quirk, G.J. (2006). Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learn. Memory *13*, 728–733.

Vouimba, R.M., Garcia, R., Baudry, M., and Thompson, R.F. (2000). Potentiation of conditioned freezing following dorsomedial prefrontal cortex lesions does not interfere with fear reduction in mice. Behav. Neurosci. *114*, 720–724.

Vouimba, R.-M., and Maroun, M. (2011). Learning-induced changes in mPFC-BLA connections after fear conditioning, extinction, and reinstatement of fear. Neuropsychopharmacology *36*, 2276–2285.

W

Wang, H.S., Pan, Z., Shi, W., Brown, B.S., Wymore, R.S., Cohen, I.S., Dixon, J.E., and McKinnon, D. (1998). KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. Science *282*, 1890–1893.

Wang, Z., Xu, N.-L., Wu, C.-P., Duan, S., and Poo, M.-M. (2003). Bidirectional changes in spatial dendritic integration accompanying long-term synaptic modifications. Neuron *37*, 463–472.

Watanabe, S., Hoffman, D.A., Migliore, M., and Johnston, D. (2002). Dendritic K+ channels contribute to spike-timing dependent long-term potentiation in hippocampal pyramidal neurons. Proc. Natl. Acad. Sci. USA *99*, 8366–8371.

Waters, J., Larkum, M., Sakmann, B., and Helmchen, F. (2003). Supralinear Ca2+ influx into dendritic tufts of layer 2/3 neocortical pyramidal neurons in vitro and in vivo. J. Neurosci. *23*, 8558–8567.

Waterhouse, I.K. (1957). Effects of prefrontal lobotomy on conditioned fear and food responses in monkeys. J. Comp. Physiol. Psychol. *50*, 81–88.

Watson, J.B. and Rayner, R. (1920). Conditioned emotional reactions. J. Exp. Psychol. *3*, 1–14.

van Welie, I., van Hooft, J.A., and Wadman, W.J. (2004). Homeostatic scaling of neuronal excitability by synaptic modulation of somatic hyperpolarization-activated Ih channels. Proc. Natl. Acad. Sci. USA *101*, 5123–5128.

Wei, A.D., Gutman, G.A., Aldrich, R., Chandy, K.G., Grissmer, S., and Wulff, H. (2005). International Union of Pharmacology. LII. Nomenclature and molecular relationships of calcium-activated potassium channels. Pharmacol. Rev. *57*, 463–472.

Weiler, N., Wood, L., Yu, J., Solla, S.A., and Shepherd, G.M.G. (2008). Top-down laminar organization of the excitatory network in motor cortex. Nat. Neurosci. *11*, 360–366.

Weisskopf, M.G., Bauer, E.P., and LeDoux, J.E. (1999). L-type voltage-gated calcium channels mediate NMDA-independent associative long-term potentiation at thalamic input synapses to the amygdala. J. Neurosci. *19*, 10512–10519.

van de Werd, H.J.J.M., Rajkowska, G., Evers, P., and Uylings, H.B.M. (2010). Cytoarchitectonic and chemoarchitectonic characterization of the prefrontal cortical areas in the mouse. Brain Struct. Funct. *214*, 339–353.

Westenbroek, R.E., Merrick, D.K., and Catterall, W.A. (1989). Differential subcellular localization of the RI and RII Na+ channel subtypes in central neurons. Neuron *3*, 695–704.

Williams, S.R., and Stuart, G.J. (2000a). Site Independence of EPSP Time Course Is Mediated by DendriticI h in Neocortical Pyramidal Neurons. J. Neurophysiol. *83*, 3177–3182.

Williams, S.R., and Stuart, G.J. (2000b). Backpropagation of physiological spike trains in neocortical pyramidal neurons: implications for temporal coding in dendrites. Journal of Neuroscience *20*, 8238–8246.

Williams, S.R., and Stuart, G.J. (2003a). Role of dendritic synapse location in the control of action potential output. Trends Neurosci. *26*, 147–154.

Williams, S.R., and Stuart, G.J. (2003b). Voltage- and site-dependent control of the somatic impact of dendritic IPSPs. J. Neurosci. *23*, 7358–7367.

Wiltgen, B.J. (2006). Context Fear Learning in the Absence of the Hippocampus. J. Neurosci. *26*, 5484–5491.

Wiltgen, B.J., and Silva, A.J. (2007). Memory for context becomes less specific with time. Learn. Memory *14*, 313–317.

X

Xu, N.-L., Harnett, M.T., Williams, S.R., Huber, D., O'Connor, D.H., Svoboda, K., and Magee, J.C. (2012). Nonlinear dendritic integration of sensory and motor input during an active sensing task. Nature *492*, 247–251.

Xu, J., Kang, N., Jiang, L., Nedergaard, M., and Kang, J. (2005). Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. J. Neurosci. *25*, 1750–1760.

Y

Yamada, S.I. (2004). Small-Conductance Ca2+-Dependent K+ Channels Are the Target of Spike-Induced Ca2+ Release in a Feedback Regulation of Pyramidal Cell Excitability. J. Neurophysiol. *91*, 2322–2329.

Yasuda, R., Sabatini, B.L., and Svoboda, K. (2003). Plasticity of calcium channels in dendritic spines. Nat. Neurosci. *6*, 948–955.

Young, S.L., Bohenek, D.L., and Fanselow, M.S. (1994). NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: immunization against amnesia by context preexposure. Behav. Neurosci. *108*, 19–29.

Yuan, L.-L., Adams, J.P., Swank, M., Sweatt, J.D., and Johnston, D. (2002). Protein kinase modulation of dendritic K+ channels in hippocampus involves a mitogen-activated protein kinase pathway. J. Neurosci. *22*, 4860–4868.

Yuan, L.-L. and Chen, X. (2006). Diversity of potassium channels in neuronal dendrites. Prog. Neurobiol. *78*, 374–389.

Z

Zelikowsky, M., Bissière, S., Hast, T.A., Bennett, R.Z., Abdipranoto, A., Vissel, B., and Fanselow, M.S. (2013). Prefrontal microcircuit underlies contextual learning after hippocampal loss. Proc. Natl. Acad. Sci. USA *110*, 9938–9943.

Zhang, Y., Fukushima, H., and Kida, S. (2011). Induction and requirement of gene expression in the anterior cingulate cortex and medial prefrontal cortex for the consolidation of inhibitory avoidance memory. Mol. Brain *4*, 4.

Zhang, F., Gradinaru, V., Adamantidis, A.R., Durand, R., Airan, R.D., de Lecea, L., and Deisseroth, K. (2010). Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. Nat. Protoc. *5*, 439–456.

Zhang, W., and Linden, D.J. (2003). The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nat. Rev. Neurosci. *4*, 885–900.

Zhang, F., Vierock, J., Yizhar, O., Fenno, L.E., Tsunoda, S., Kianianmomeni, A., Prigge, M., Berndt, A., Cushman, J., Polle, J., et al. (2011). The microbial opsin family of optogenetic tools. Cell *147*, 1446–1457.

Zhao, M.-G., Toyoda, H., Lee, Y.-S., Wu, L.-J., Ko, S.W., Zhang, X.-H., Jia, Y., Shum, F., Xu, H., Li, B.-M., et al. (2005). Roles of NMDA NR2B Subtype Receptor in Prefrontal Long-Term Potentiation and Contextual Fear Memory. Neuron *47*, 859–872.

Zhou, Y.-D., Acker, C.D., Netoff, T.I., Sen, K., and White, J.A. (2005). Increasing Ca2+ transients by broadening postsynaptic action potentials enhances timing-dependent synaptic depression. Proc. Natl. Acad. Sci. USA *102*, 19121–19125.

Zhou, Y., Won, J., Karlsson, M.G., Zhou, M., Rogerson, T., Balaji, J., Neve, R., Poirazi, P., and Silva, A.J. (2009). CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. Nature Neurosci. *12*, 1438–1443.

Appendix

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Chapter 2

Plasticity and Pathology of Dendritic Intrinsic Excitability

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Abstract

The dendrites of most CNS neurons integrate synaptic signals from hundreds-to-thousands of neurons from a variety of brain regions to give rise to neuron-type specific action potential output. These complex computations are determined by the excitability (active properties) of dendrites due to the expression of myriads of voltage-gated ion channels in their membrane. The properties and expression levels of these ion channels in the membrane are characteristic for a given neuron type and their sub-cellular compartments (e.g., proximal versus distal dendrites). Thereby, the intrinsic excitability of dendrites determines the rules for plasticity and computation. Any change in the expression pattern or biophysical properties of these voltage-gated ion channels will alter the intrinsic excitability of neurons. Plasticity of intrinsic excitability—henceforth called *intrinsic* plasticity—can be triggered by various neuromodulators, activity patterns, disease states, or learning paradigms. The functional consequences of intrinsic plasticity for neuronal functioning will depend on the nature of the change (e.g., increase or decrease in excitability), its cellular localisation (e.g., neuron-wide or global versus individual dendritic branch), and its time course (transient, long-term). There is increasing evidence linking this form of plasticity to processes such as memory formation and disease. In this chapter, we will give an overview of the repertoire of ion channels in the dendrites of mammalian neurons, and discuss the concept of dendritic intrinsic

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excitability, its plastic modulation, and its pathological alteration in a number of CNS disorders.

Introduction

The traditional view of dendrites is that they are merely passive structures that collect synaptic inputs to transfer them to the soma. Over the past 20 years or so it has become clear that dendrites have active properties, shaping information transfer along the dendritic tree. These active properties enable dendrites to perform local computations such as the generation of spikes, and they are required for certain forms of synaptic and non-synaptic plasticity. Most neuron types possess elaborate dendritic arbours that receive and integrate excitatory and inhibitory inputs from numerous other neurons to give rise to cell-type specific firing patterns. The computational properties of these dendrites are therefore crucial for information processing within the neuron.

Dendrites contain a great variety of voltage-gated sodium (Na^+), calcium (Ca^{2+}), potassium (K^+) and hyperpolarization-activated and cyclic nucleotide-gated (h) channels, as well as voltage-independent ion channels, which play an important role in determining the excitability of the neuron either locally or globally (Migliore and Shepherd, 2002; Magee, 2008; Nusser, 2008; Nusser, 2012). The properties and expression levels of these channels are characteristic for a given neuron type and their sub-cellular compartments (e.g., soma versus dendrites). As such, the repertoire and subcellular distribution pattern of ion channels expressed constitutes a major factor determining the functional heterogeneity of neuron types (Nusser, 2012). Any change in the subcellular distribution, density, biophysical properties, or activity level of these voltage-gated ion channels will alter the intrinsic excitability of neurons (Zhang and Linden, 2003; Frick and Johnston, 2005; Remy et al., 2010). Henceforth, the plasticity of intrinsic excitability is called *intrinsic* plasticity to distinguish it from *synaptic* plasticity. Dendritic intrinsic plasticity can be triggered by various neuromodulators, activity patterns, disease states, or learning paradigms (reviewed in Zhang and Linden, 2003; Frick et al., 2004; Frick and Johnston, 2005; Disterhoft and Oh, 2006; Benito and Barco, 2010; Mozzachiodi and Byrne, 2010).

We will begin our discussion by describing the ion channels implicated in the regulation of active dendritic properties. We will then move onto discussing the plasticity of dendritic excitability. In the last part of the chapter we will review examples of disorders linked to pathological changes in ion channel function and/or expression. For simplicity, we will discuss mainly studies performed on mammals with particular focus on what is known about dendritic excitability in the hippocampus, neocortex and cerebellum. For more information about changes in global intrinsic excitability (Benito and Barco, 2010; Mozzachiodi and Byrne, 2010) as well as studies on invertebrates please refer to Laurent and Borst (2008).

The Dendritic Ion Channel Landscape

Voltage-dependent ion channels are typically composed of monomeric or tetrameric pore-forming transmembrane proteins with a specific selectivity for certain ions (Na^+, Ca^{2+}, K^+, or Cl^-). These pore-forming subunits are associated with auxiliary subunits, as well as a

range of other molecules, such as scaffolding proteins, which serve to modulate their properties or subcellular location (reviewed in Vacher et al., 2008; Zamponi and Currie, 2013; Leterrier et al., 2010; Jensen et al., 2011; Lai and Jan, 2006; Gutman et al., 2005). Differential splicing and editing of their mRNAs, as well the formation of heteromers and additional post-translation modifications at the protein level further extends their diversity (reviewed in Cerda and Trimmer, 2010; Vacher et al., 2008; Jan and Jan, 2012; Lipscombe et al., 2013; Zamponi and Currie, 2013; Wahl-Schott and Biel, 2009). Voltage-gated ion channels are classified into a number of broad categories based on their selectivity for certain ions, for example K^+, Na^+, Ca^{2+}, or their activation by hyperpolarisation (i.e., hyperpolarisation activated and cyclic nucleotide-gated channels). For the purposes of this review, we will, for the most part, limit our discussion to these major classes, which form the majority of channel types present in mammalian dendrites.

Voltage-Gated Na^+ Channels

Voltage-gated Na^+ channels (Na_v subunits) are essential for the fast influx of Na^+ ions underlying membrane depolarisation, thereby increasing membrane excitability (Hille, 2001). Their activation permits the active backpropagation of action potentials (AP) into the dendrite, the genesis of Na^+-mediated dendritic spikes and the amplification of synaptic potentials (reviewed in Magee, 2008).

Of the ten known Na_v family members, four subunits ($Na_v1.1$, 1.2, 1.3, and 1.6) are expressed in the rodent/human brain with cell-type specific subcellular expression patterns (Table 1). $Na_v1.3$ expression is thought to be limited to the embryonic and early postnatal rodent brain (reviewed in Vacher et al., 2008), however several reports suggest that it may also be expressed in the neocortex and hippocampus of young adult or adult rodents (Westenbroek, et al., 1992; Xu et al., 2013). $Na_v1.1$ subunits are expressed in the somatodendritic compartment of layer (L) 5 neocortical neurons, CA1 and CA3 pyramidal neurons, and Purkinje cells (Gong et al., 1999), as well as in the axons of hippocampal and neocortical parvalbumin-positive interneurons (Ogiwara et al., 2007). $Na_v1.6$ is expressed in the apical dendrites of L5 pyramidal neurons of the neocortex and CA1 region of the hippocampus and the dendrites of Purkinje neurons (Krzemien, et al., 2000). Studies of the exact Na_v subunits responsible for dendritic Na^+ currents are elusive due to the absence of specific inhibitors for each subtype. Recent EM-based mapping of dendritic $Na_v1.6$ in the apical dendrites of CA1 pyramidal neurons suggests that there is a gradient of these subunits along the somatodendritic axis, declining in density with distance from soma (Lörincz and Nusser, 2010). In addition, functional gradients may exist due to changes in the phosphorylation state of Na^+ channels across their dendritic arbour (reviewed in Magee, 2008). This phosphorylation (at least in CA1 pyramidal neurons) is most likely mediated through protein kinase C (PKC) and can result in a depolarised shift in the activation curve at more proximal versus distal dendritic locations (Gasparini and Magee, 2002). Another interesting phenomenon is the presence of two populations of Na^+ channels with differing inactivation kinetics, namely fast and slow (i.e., requiring seconds for complete recovery, in CA1 pyramidal neurons that appear to be differentially distributed between the dendrites and soma (Colbert et al., 1997; Jung et al., 1997; Mickus et al., 1999). The population of channels with slow inactivation kinetics is more prominent in the dendrites compared to the soma. It

has been suggested that these two populations are the result of differing phosphorylation states (Colbert and Johnston, 1998) or Na_v channels with a different molecular composition (Magee, 2008).

Mutations in the genes encoding all of the aforementioned subunits are associated with inherited epilepsy (Meisler and Kearney, 2005; Estacion et al., 2010; Oliva et al., 2012). In addition, certain disorders may lead to the inappropriate or ectopic expression of subunits not normally expressed in the brain (Schaller and Caldwell, 2003; Hains et al., 2005; Verret et al., 2012).

Table 1. Summary of CNS-specific Na_v channel subunits, their genes and subcellular localisation. Adapted from Vacher et al., 2008. The previous name of each subunit is given in parenthesis to aid comparison with prior literature

Subunit	Gene	Localisation	Representative references
$Na_v1.1$ (Type I)	*SCN1A*	Somatodendritic	*Gong* et al., *1999; Westenbroek*, et al., *1989*
$Na_v1.2$ (Type II)	*SCN2A*	Axonal, mostly limited to axon initial segment	
$Na_v1.3$ (Type III)	*SCN3A*	Somatodendritic	*Westenbroek* et al., *1992*
$Na_v1.6$ (Type VIII)	*SCN8A*	Axon initial segement, somato-dendritc	*Lörincz and Nusser, 2010; Krzemien* et al., *2000*

Voltage-Gated Ca^{2+} Channels

Voltage-gated Ca^{2+} channels (Ca_v subunits) permit dendritic Ca^{2+} transients in response to excitatory stimuli in the dendrite (Hille, 2001; Berridge, 1998; Catterall, 2000). Low-voltage activated Ca^{2+} channels (T-type) are activated by subthreshold postsynaptic potentials, whereas high-voltage activated Ca^{2+} channels are opened by backpropagating APs (bAPs) and are required for the generation of dendritic Ca^{2+} spikes (Magee et al., 1995; Schiller et al., 1995; Schiller et al., 1997; Larkum, et al., 1999a; Frick et al., 2003). The Ca^{2+} influx mediated by Ca^{2+} channels not only increases dendritic excitability, but can also have a range of down-stream consequences for the neuron including the activation of Ca^{2+}-dependant signalling pathways (e.g., Dolmetsch et al., 2001) and activity-dependent gene transcription (reviewed in Catterall, 2000; Zamponi and Currie, 2013). Ca^{2+} channels thus provide a link between membrane excitability and other dynamic cellular processes.

Ca^{2+} channels are classified into five main families based on their activation/inactivation kinetics, susceptibility to antagonists (Table 2) and ability to be modulated by intracellular signalling pathways (reviewed in Catterall, 2000; Zamponi and Currie, 2013). Ca^{2+} channels are composed of a macromolecular complex comprising a pore-forming ($\alpha s1$)-subunit, an $\alpha s2$- and a β-subunit, as well as several auxiliary subunits and calmodulin (reviewed in Zamponi and Currie, 2013; Vacher et al., 2008). The $\alpha s1$-subunit is a single 24-transmembrane domain comprising four pseudosubunits, which form the channel pore, as well as N-terminal and C-terminal cytoplasmic domains, which are the targets of regulatory

molecules. The cytoplasmic domains are, for example, targets for modulation by G-protein coupled receptors and intracellular signalling pathways, such as Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII; reviewed in Zamponi and Currie, 2013, Catterall et al., 2000; Vacher et al., 2008).

All five Ca^{2+} channel types have some dendritic expression. L-type, R-type and T-type Ca^{2+} channels appear to be exclusively somatodendritic (reviewed in Vacher et al., 2008). N- and P/Q-type channels, although present in dendrites, are typically expressed at much higher densities in the axon (Johnston and Narayanan, 2008). In spite of these broad rules, the expression of individual subunits within a family can vary markedly. For example, the T-type subunit $Ca_v3.1$ is primarily limited to the soma and proximal dendrites of pyramidal neurons in a range of brain areas, whereas $Ca_v3.3$ is expressed along the entire length of the dendrite (in addition to the soma) in the same neurons. $Ca_v3.2$ shows a pattern of distribution that is somewhat intermediate between the two (McKay et al., 2006).

Ca^{2+} currents of all major types have been detected in the dendrites of many CNS neurons (reviewed in Magee, 2008; Johnston and Narayanan, 2008). These can be distinguished on the basis of their voltage-dependence and sensitivity to a number of specific inhibitors/antagonists (reviewed in Magee, 2008), however, the individual subunits responsible for these currents cannot be distinguished in this manner (Johnston and Narayanan, 2008). Mutations in Ca_v channel-encoding genes have been implicated in a number of neurological disorders (reviewed in Catterall, 2011).

K^+ Channels

K^+ channels are crucial regulators of membrane excitability. These channels represent the most diverse group of ion channels and can be further subdivided into several groups based on their pore-forming α-subunits (Luján, 2010; Johnston and Narayan, 2008). These α-subunits can be classified into voltage-dependent (K_v) subunits, inward rectifier (K_{ir}) subunits, two-pore (K_{2P}) subunits, subunits activated by intracellular Ca^{2+} (K_{Ca}), and those activated by intracellular Na^+ (K_{Na}).

Voltage-Gated K^+ Channels

Voltage-gated K^+ channels (K_v subunits) represent the largest and most diverse group of voltage-gated ion channels (Vacher et al., 2008; Luján, 2010). Their α-subunits consists of one six-transmembrane pore-forming domain that co-assembles with three other α-subunits to form the tetrameric pore-forming domain (reviewed in Vacher et al., 2008). This permits the formation of heteromeric pore-forming subunits, increasing the molecular diversity of K^+ channels (reviewed in Vacher et al., 2008). Further functional diversity is achieved through the association of the pore-forming subunits with various β-subunits (reviewed in Vacher and Trimmer, 2011; Pongs and Schwarz, 2010).

Table 2. Summary of characteristics and major alpha contributing subunit of Ca_v channel subtypes. Adapted from Vacher et al., 2008; Catterall WA, 2000; Perez-Reyes and Schneider, 1994

Channel type	αs1 subunit	Gene name	Activation threshold	Deactivation rate	Inactivation rate	Subcellular location	Specific agonist
L-type	$Ca_v1.2$ $Ca_v1.3$	*CACNA1C* *CACNA1D*	High	Fast	Slow	Soma, dendrites, spines	Dihydro-pyridine (DHP)
P/Q-type	$Ca_v2.1$	*CACNA1A*	High	Fast	Very slow or moderate	Axons, soma and dendrites	ω-Agatoxin
N-type	$Ca_v2.2$	*CACNA1B*	High	Fast	Moderate	Axons, soma and dendrites	ω-CTx-GV1A
R-type	$Ca_v2.3$	*CACNA1E*	High	Fast	Fast	Soma and dendrites	None
T-type	$Ca_v3.1$ $Ca_v3.2$ $Ca_v3.3$	*CACNA1G* *CACNA1H* *CACNA1I*	Low	Slow	Fast	Soma and dendrites	None

Table 3. Summary of characteristics and major alpha subunits of dendritic K_v channel subtypes. Adapted from Vacher et al., 2008

Subunit	Gene name	Type of current	*Drosophila homologue*	Cellular localisation	Commentary
$K_v1.1$	KCNA1	Transient	*Shaker*	Axon, some dendritic	
$K_v2.1$	KCNB1	Inward rectifier	*Shab*	Mostly proximal dendrites and soma	Localisation regulated by activity[1]
$K_v2.2$	KCNB2	Inward rectifier	*Shab*	Soma and dendrites	
$K_v3.1$	KCNC1	Sustained K^+ current	*Shaw*	Axonal or somatodendritic	Localisation depends on splice form[2]
$K_v3.2$	KCNC2	Sustained K^+ current	*Shaw*	Soma, dendrites	
$K_v4.2$	KCND2	A-current	*Shal*	Somatodendritic	Membrane localisation regulated by activity[3]
$K_v4.3$	KCND3	A-current	*Shal*	Somatodendritic	
$K_v7.2$	KCNQ2	M-current	none	Mostly axonal, some somato dendritic	Detected in dendrites in caudate putamen[4] and parvalbumin-positive neurons of dentate gyrus [5]
$K_v7.3$	KCNQ3	M-current	none	Axons, soma, dendrites	
$K_v7.5$	KCNQ5	M-current	none	Soma, dendrites	

[1]Misonou et al., 2006
[2]Ozaita et al., 2002
[3]Kim et al., 2007
[4]Cooper et al., 2001
[5]Nieto-Gonzalez and Jensen, 2013

A summary of the major dendritically localised K_v α-subunits is presented in Table 3. Since the prototypic K_v subunits were initially identified by mutant screens in *Drosophila*, rather than by biochemical analysis, they are often known by the name of their *Drosophila* homologue (i.e., *Shaker, Shab, Shaw, Shal*). Within neurons, these channels fulfil numerous functions, for example, as generators of sustained currents ($K_v1.1$, $K_v3.1$, $K_v3.2$), delayed rectifying currents ($K_v2.1$, $K_v2.2$) or transient A-type currents ($K_v1.4$, $K_v4.2$, $K_v4.3$). The K_v7 subfamily (a separate group of channels with limited homology to the other K_v subunits) comprises the channels responsible for M-current (Luján, 2010).

Arguably, the most studied members of the dendritic K_v family are the channels responsible for the rapidly activating and inactivating A-type K^+ current (composed of the subunits $K_v4.2$ and $K_v4.3$). $K_v4.2$ is regulated by a number of intracellular signalling pathways and kinases, including the extracellular-signal-regulated kinase (ERK), PKC and CaMKII, and has been implicated in numerous plasticity paradigms as well as several pathologies (e.g., Schrader et al., 2009; Lugo et al., 2008; Varga et al., 2004; Gross et al., 2011; Lockridge and Yuan, 2011; Lugo et al., 2012; Morozov et al., 2003; see also below). It has been suggested that K_v4 channels play a role in synaptic integration, modulation of backpropagating APs, and in the gating of dendritic spiking in response to diverse inputs arriving from different sources, in neocortical and hippocampal neurons (Hoffman et al., 1997; Frick et al., 2003; Burkhalter et al., 2006). This is exemplified by the pronounced gradient of A-type current recorded along the apical dendrites of CA1 pyramidal neurons (Hoffman et al., 1997), or the compartmentalised expression of both $K_v4.2$ and $K_v4.3$ subunits observed in the primary visual cortex (Burkhalter et al., 2006). Interestingly, in the apical dendrites of CA1 neurons, this gradient of current is not reflected in a similar gradient of $K_v4.2$ protein and is likely mediated through other means such as modulation or modification of the α-subunits or auxiliary subunits (Kerti et al., 2012; see also Vacher and Trimmer, 2011).

Other K^+ Channels

Numerous other (non K_v-type) K^+ channels may also play a role in dendritic physiology, such as the inward rectifier subfamily (K_{ir}) that includes the G-protein-coupled inwardly rectifying K^+ (GIRK; also known as $K_{ir}3$) channels, the two-pore domain K^+ channels (K_{2P}), members of the Ca^{2+}-dependent K^+ channel subfamily (K_{Ca}; comprising the BK and SK channels), and members of the Na^+-dependent K^+ channel subfamily (K_{Na}; comprising the Slack and Slick channels). GIRK channels, for instance, are voltage-independent and activated by binding of G protein subunits, resulting in long-lasting membrane hyperpolarisations (Luján et al., 2009). The two-pore domain K^+ channels provide a leak current that contributes to the resting membrane potential and suppresses membrane excitability. In dendrites, this current may reduce the summation of excitatory postsynaptic potentials (EPSPs; Day et al., 2005). Ca^{2+}-/Na^+-dependent K^+ channels require changes in the concentration of intracellular ions (BK, SK: Ca^{2+}; Slack, Slick: Na^+) for their activation, either in a voltage-dependent (e.g., BK channels) or -independent (e.g., SK channels) manner. Ca^{2+}-dependent K^+ channels contribute to the fast/slow afterhyperpolarisation during trains of APs, thereby regulating the re-polarisation of APs and the neuronal firing pattern. BK channels are also important regulators of dendritic excitability by reducing AP backpropagation efficacy and increasing the threshold for dendritic Ca^{2+} spikes (Benhassine

and Berger, 2009). Na^+-dependent K^+ channels contribute to the AP repolarisation, the slow afterhyperpolarisation following repetitive firing, and the firing rate adaptation (reviewed in Bhattacharjee and Kaczmarek, 2005).

Hyperpolarisation-Activated and Cyclic Nucleotide-Gated Channels (H Channels)

Hyperpolarisation–activated and cyclic nucleotide-gated channels (HCN) are the molecular correlate of I_h (the current mediated by h channels). I_h exerts a diverse range of functions in the dendrites. It contributes to the resting membrane potential, acts as a resonator conductance, suppresses temporal and spatial summation of synaptic input, and decreases the efficacy of backpropagating APs (Berger et al., 2001; Williams and Stuart, 2000b; Chevaleyre and Castillo, 2002; Kole et al., 2006; Tsay et al., 2007; Angelo et al., 2007; Brager and Johnston, 2007; Zemankovics et al., 2010). Most of its actions reduce dendritic excitability and the impact of synaptic input in driving the AP output of neurons. On the other hand, I_h causes rebound depolarisations following membrane hyperpolarisations that contribute to the generation of dendritic plateau potentials or spikes (Aizenman and Linden, 1999; Engbers et al., 2011).

H channels are composed of four pore-forming 'alpha'-like subunits with similar structural organisation to the alpha units of K_v channels (Luján, 2010). These pore-forming domains are also associated with auxiliary subunits, which in part determine the localisation and active properties of these channels and also provide an interface with cellular signalling pathways (reviewed in Wahl-Schott and Biel, 2009; Biel et al., 2009; Lewis et al., 2010). HCN1 and HCN2 are the two major subunits responsible for I_h in neocortex and hippocampus (Notomi and Shigemoto, 2004), where they are primarily localised to the dendrites. The other subunits (HCN3 and HCN4) may be the predominant subunits in other brain regions, but their subcellular expression has not been studied in detail. In the neocortex and hippocampal CA1 region, HCN1 and HCN2 co-localise and are thus thought to form hetero-tetramers, however, HCN1 and HCN2 exhibit different patterns of expression in early postnatal life (Brewster et al., 2007). H channels are also expressed in interneurons. Although their expression appears very sparse in neocortical interneurons, HCN1 immunoreactivity has been observed in hippocampal parvalbumin-positive interneurons (Lörincz et al., 2002; Brewster et al., 2002). I_h has been measured in the dendrites of both L5 pyramidal neurons of the neocortex and CA1 pyramidal neurons of the hippocampus. In both cases I_h exhibits a dramatic gradient with densities of this current increasing along the length of the apical dendrite, up to the major branch point (Magee, 1999; Williams and Stuart, 2000b). A recent study examining this current in the distal dendrites of CA1 neurons suggest this dramatic gradient does not, however, extend into the distal tuft region in CA1 pyramidal neurons (Bittner et al., 2012). This contrasts with immunohistochemical data (Notomi and Shigemoto, 2004) showing a continuation of this gradient into the distal tuft and furthermore suggests a different integrative mechanism in the tuft (Bittner et al., 2012). The function of this variation in current densities may be to permit scaling of different synaptic inputs from vastly different sources over the different strata of the hippocampus. Furthermore, evidence suggests that appropriately timed spatially segregated excitatory input is critical for the correct development of this gradient of I_h (Shin and Chetkovich, 2007). In the neocortex, the density

of functional h channels present in the membranes of distal dendrites increases in a developmentally-specific manner from early postnatal life to young adulthood, although the density of proximal channels is unchanged (Atkinson and Williams, 2009). I_h thus plays a role in the developmental maturity of dendritic properties. Additionally, I_h exerts a diverse range of functions in the dendrite from the summation of synaptic input to its coupling to the AP output of neurons. Dysfunction of h channels has been linked to a number of disorders, most notably epilepsy and certain pain disorders (reviewed in Poolos and Johnston, 2012; Benarroch, 2013).

Plasticity of Dendritic Excitability

Any form of cognitive, sensory or motor processing requires neurons to integrate physiologically relevant incoming synaptic information and to convert it into a pattern of precisely timed AP output. To accomplish this, neuronal dendrites continuously 'evaluate' the strength, spatial and temporal distribution of synaptic potentials, as well as govern the efficacy with which these signals are propagated. They also give rise to local regenerative events, determine the AP output at the soma and its active backpropagation back into the dendrites (Frick and Johnston, 2005; London and Häusser, 2005; Spruston, 2008; Johnston and Narayanan, 2008). As already discussed, the fundamental mechanism allowing dendrites to perform their complex computations is the specific subcellular distribution and biophysical properties of voltage-gated ion channels.

The manner in which information is processed and conveyed has important functional consequences, both at the exact time of neuronal activation as well as in the future. That is why another critical aspect of dendritic function is to govern various forms of synaptic and non-synaptic plasticity. Neural plasticity is most frequently studied as a change in the efficacy of synaptic transmission. This is because the number of potentiated synapses, their local clustering and temporal pattern of activation are all thought to underlie information transfer and storage (Mayford et al., 2012). However, neuronal plasticity encompasses more than just chemical and morphological modifications at the level of the synapse. This dynamic process also includes the remodelling of neuronal circuits (Chklovskii et al., 2004), neurogenesis (Deng et al., 2010; Marín-Burgin and Schinder, 2012), and changes in intrinsic excitability (Zhang and Linden, 2003; Frick and Johnston, 2005; Mozzachiodi and Byrne, 2010). We now know that intrinsic excitability plays an important role in behavioural learning and in the processing of sensory/motor stimuli. Additionally, it has been suggested to serve as a mechanism for metaplasticity, memory allocation or even part of the memory trace itself (Helmchen et al., 1999; Xu et al., 2012; Zhang and Linden, 2003; Frick et al., 2005; Zhou et al., 2009; Mozzachiodi and Byrne, 2010).

In this section we review some of the basic mechanisms of dendritic information processing as well as what is known about the plasticity of dendritic excitability. We also touch upon the interaction between dendritic excitability and synaptic plasticity. Wherever possible, we attempt to draw links between behavioural/*in vivo* studies and dendritic plasticity.

Action Potential Backpropagation

APs are electrical events, usually initiated in the axo-somatic region that travel forward along the axon but that can also propagate back into the dendrites (Stuart et al., 1997b). AP backpropagation can occur passively, being shaped by the passive electrical properties of the neuron (Stuart et al., 1997b) and its dendritic morphology (Vetter et al., 2001), but also actively, due to the presence of voltage-gated Na^+ channels expressed along the dendritic membrane (Stuart and Sakmann, 1994; Johnston et al., 1996; Stuart et al., 1997b). The efficacy of AP backpropagation is highly variable between different neuronal types (for reviews see Stuart et al., 1997b; Sjöström et al., 2008). For example, in cerebellar Purkinje neurons, where the density of dendritic Na^+ channels is low, the backpropagation of APs is mostly passive. This results in significant voltage attenuation as the AP spreads throughout the dendritic tree (Stuart and Häusser, 1994). On the other side of the spectrum, in the dopaminergic neurons of substantia nigra, where backpropagation is highly active, the distance dependent voltage attenuation is very small (Häusser et al., 1995; Gentet and Williams, 2007). In pyramidal neurons of neocortical L5 (Stuart and Sakmann, 1994; Nevian et al., 2007; Grewe et al., 2010), L2/3 (Waters et al., 2003) and hippocampal CA1 (Spruston et al., 1995; Golding et al., 2001) AP backpropagation efficacy can be placed in the intermediate range. Consequently, the potential spread is active but may also be incomplete failing to invade distal dendritic branches. AP backpropagation critically depends on the presence of Na^+ channels, however the degree and manner of this retrograde propagation is sensitive to the activity of a range of voltage-gated ion channels (Colbert et al., 1997; Hoffman et al., 1997; Jung et al., 1997; Migliore et al., 1999; Berger et al., 2003), prior neuronal activity (Spruston et al., 1995; Magee and Johnston, 1997; Stuart and Häusser, 2001; Sjöström and Häusser, 2006), and neuromodulation (Häusser et al., 1995; Hoffman and Johnston, 1999; Gentet and Williams, 2007). BAPs are known to occur both *in vitro* (Stuart and Sakmann, 1994; Spruston et al., 1995; Nevian et al., 2007; Grewe et al., 2010) and *in vivo* (Buzsáki et al., 1996; Waters et al., 2003; Bereshpolova et al., 2007).

AP backpropagation plays an important role in the regulation of dendritic excitability. It provides feedback information into the dendrites about axonal output, causes postsynaptic depolarisation and triggers Ca^{2+} influx into the cell through the activation of voltage-gated Ca^{2+} channels, creating an important link between electrical events and Ca^{2+}-dependent intracellular signalling cascades (Jaffe et al., 1992; Christie et al., 1995; Frick et al., 2003; Waters et al., 2003). When bAPs are paired with incoming EPSPs the result is a supralinear Ca^{2+} influx into the cell, which further increases dendritic excitability and facilitates synaptic integration (Waters et al., 2003; Frick and Johnston, 2005; Sjöström et al., 2008). If the amount of the resulting dendritic depolarisation is sufficient, the generation of dendritic Ca^{2+} spikes can in turn evoke burst firing at the soma, a phenomenon called BAC firing (Larkum et al., 1999b). When appropriately timed with EPSPs, bAPs can also induce long-term potentiation (LTP) and depression (LTD) through spike-timing-dependent-plasticity protocols (STDP; Magee and Johnston, 1997, Letzkus et al., 2006; Sjöström and Häusser, 2006; for review on STDP see Dan and Poo, 2006).

Dendritic Spikes

The synchronous activation of multiple spatially clustered synapses can result in large transient depolarisations, reflecting the opening of voltage-gated Na^+ and Ca^{2+} channels. This locally generated supralinear membrane event is called a dendritic spike (Häusser et al., 2000; Gulledge et al., 2005; Major et al., 2013). In some dendrites, the activation of voltage and ligand-gated N-methyl-D-aspartate (NMDA) receptors can result in an NMDA spike (Schiller et al., 2000; Schiller and Schiller, 2001; Rhodes, 2006; Larkum et al., 2009; Major et al., 2013). Dendritic spikes were first observed in cerebellar Purkinje neurons (Llinás and Sugimori, 1980) and in hippocampal pyramidal cells (Wong et al., 1979; Benardo et al., 1982). Now, it is now known that dendritic spikes occur in many different cell types (Amitai et al., 1993; Chen and Shepherd, 1997; Larkum et al., 1999a; Martina et al., 2000; Goldberg et al., 2004; Kitamura and Häusser, 2011; Katona et al., 2011) both *in vitro* and *in vivo* (Helmchen et al., 1999; Waters et al., 2003; Larkum et al., 2007; Kitamura and Häusser, 2011; Xu et al., 2012; Lavzin et al., 2012; Losonczy et al., 2008; Makara et al., 2009). In pyramidal neurons, Na^+ channels mediate dendritic spikes at proximal locations (Amitai et al., 1993; Ariav et al., 2003; Nevian et al., 2007), whereas the more distal spikes are generally mediated by voltage-gated Ca^{2+} channels (Schiller et al., 1997; Stuart et al., 1997b; Larkum et al., 1999b). NMDA spikes are most likely to occur in basal (Schiller et al., 2000; Nevian et al., 2007) and tuft dendrites (Larkum et al., 2009) and they can play an important role in the supralinear integration of synaptic inputs (Mel, 1993; Polsky et al., 2004; Larkum et al., 2009).

Dendritic spikes are regenerative events because they arise from membrane depolarisation and cause further depolarisation (Larkum et al., 1999a; Häusser et al., 2000). They are also heterogeneous in nature, with some spreading efficiently towards the soma (Chen and Shepherd, 1997; Martina et al., 2000; Larkum and Zhu, 2002) and others remaining constricted to the local dendritic area (Schiller et al., 1997; Golding and Spruston, 1998; Losonczy and Magee, 2006; Losonczy et al., 2008). Strong somatic stimulation above a particular frequency, called the critical frequency, can result in local electrogenesis in the form of dendritic Ca^{2+} spikes (Stuart et al., 1997a; Larkum et al., 1999a; Williams and Stuart, 2000a; Berger et al., 2003; Larkum et al., 2007). The critical frequency threshold can be influenced by changes in the dendritic membrane potential and depends on the presence of I_h currents (Berger et al., 2003). If dendritic spikes are large enough they can trigger somatic APs and burst firing (Golding and Spruston, 1998; Williams and Stuart, 1999; Larkum et al., 1999b; Larkum and Zhu, 2002). Dendritic spikes play an important role in sensory processing as well as in the timed integration of synaptic inputs (Helmchen et al., 1999; Xu et al., 2012; Lavzin et al., 2012; Breton and Stuart, 2009).

Synaptic Integration

The excitability of a neuron determines its propensity to generate an AP from incoming synaptic inputs. The spatial and temporal pattern of synaptic activation is therefore critical for determining the neuronal output, not only at the precise time of activation but also in the future. The large distribution of synaptic inputs together with the cable filtering properties of dendrites could however decrease the precision with which a neuron can determine and

integrate incoming synaptic signals. This could in turn increase the variability of AP firing and decrease the effectiveness of temporal coincidence detection (Magee, 2000). Early data from intracellular recordings and neuronal models suggested that a significant attenuation of synaptic potentials occurred between the site of initiation and the soma (Stuart et al., 1997b; Williams and Stuart, 2003b). We now know, however, that dendrites possess active mechanisms that can reduce the dependence of synaptic effectiveness on input location (Johnston et al., 1996; Yuste and Tank, 1996; Magee, 2000; Gulledge et al., 2005; Sjöström et al., 2008).

EPSP summation can be influenced by dendritic Na^+, A-type K^+ and Ca^{2+} channels (Lipowsky et al., 1996; Schwindt and Crill, 1996; 1997; Cash and Yuste, 1998; 1999; Magee and Johnston, 2005) but h channels seem to be of particular importance for subthreshold integration. This is due to their specific activation kinetics as well as their subcellular gradient of distribution. In pyramidal neurons of CA1 and neocortical L5, these channels are distributed non-uniformly and their density is increased severalfold in distal dendrites when compared to proximal dendrites (Magee 1998; 1999; Lörincz et al., 2002; Williams and Stuart, 2000b). They are activated by membrane hyperpolarisation but result in membrane depolarisation (Robinson and Siegelbaum, 2003). At depolarised membrane potentials these channels are deactivated, which effectively results in a hyperpolarising outward current occurring during synaptic activity. Consequently, the activation of h channels reduces the duration of inhibitory postsynaptic potentials (IPSPs; Williams and Stuart, 2003a), whereas their deactivation reduces the duration of EPSPs (Magee, 1998; 1999). Because of the specific distribution gradient of h channels, their activity has strongest consequences for the temporal/spatial summation of distal synaptic inputs. This distance-dependent normalisation of temporal summation overcomes the passive filtering of neuronal dendrites causing synaptic potentials measured at the soma to sum similarly regardless of the input location (Magee, 1999; 2000).

Activity-Induced Plasticity of Dendritic Excitability

Previous neuronal firing activity, local membrane depolarisations or the interaction of the two can significantly alter the efficacy of AP backpropagation, dendritic spike generation and synaptic integration. For example in neocortical L5 (Stuart and Sakmann, 1994) and in hippocampal CA1 neurons (Spruston et al., 1995; Golding et al., 2001) a train of APs induced in the soma causes a reduction in the amplitude of bAPs when measured along the apical dendrite. This decrease in amplitude follows a slow, voltage-dependent slope of recovery and is coupled with a significant decrease in dendritic Ca^{2+} transients (Jaffe et al., 1992; Callaway and Ross, 1995; Spruston et al., 1995). Such long-lasting voltage attenuation is due to a prolonged inactivation of dendritic Na^+ channels (Stuart and Sakmann, 1994; Jung et al., 1997) in CA1 pyramidal neurons, and also to the activity of A-type K^+ channels (Colbert et al., 1997; Hoffman et al., 1997).

Voltage-dependent A-type K^+ channels play a key role in regulating the amplitude of bAPs (Hoffman et al., 1997; Migliore et al., 1999; Frick et al., 2003; Martina et al., 2003). These rapidly activating and inactivating channels provide transient outward currents and are present in high densities in the dendrites of pyramidal neurons (reviewed in Sjöström et al., 2008; Spruston, 2008). The pharmacological block of A-type K^+ channels by 4-

Aminopyridine (4-AP) increases dendritic AP amplitude in a dose-dependent manner (Hoffman et al., 1997). The deletion of the *K_v4.2* gene almost completely eliminates A-type K^+ currents from CA1 pyramidal neurons leading to an increase in bAP amplitude and the associated Ca^{2+} influx (Chen et al., 2006). K_v4.2 has phosphorylation sites for protein kinase A (PKA), PKC, CaMKII and mitogen-activated protein kinase (MAPK; Adams et al., 2000; Anderson et al., 2000; Yuan et al., 2002). The activation of these kinases by the neurotramsitters glutamate (via mGluRs), dopamine, noradrenaline and acetocholine decreases the activity of these channels and leads to an increase in bAP amplitude (Hoffman and Johnston, 1998; 1999).

Dendritic membrane depolarisation and synaptic activity can also overcome bAP amplitude attenuation through a rise in intracellular Ca^{2+} mediated by CaMKII (Magee and Johnston, 1997; Stuart and Häusser, 2001; Sjöström and Häusser, 2006; Tsubokawa et al., 2000). When bAPs are paired with subthreshold EPSPs, the resulting dendritic depolarisation promotes the recruitment of Na^+ channels and/or K^+ channel inactivation causing a facilitation of AP backpropagation (Hoffman et al., 1997; Migliore et al., 1999; Stuart and Häusser, 2001; Gasparini et al., 2007). Moreover, synaptic input that is precisely timed with AP firing in an STDP protocol increases bAP amplitude in the potentiated dendritic region via a change in A-type K^+ currents (Frick et al., 2004).

The activity-dependent modulation of bAP amplitude may have important behavioural relevance. Quirk et al. (2001) showed that as rats gained experience of a given environment there was an experience-dependent decrease in bAP amplitude attenuation (Quirk et al., 2001). In mitral cells in the olfactory bulb, repetitive somatic stimulation mimicking odour-induced activity causes an enhancement of AP backpropagation into the distal lateral dendrites. This is mediated by a supralinear increase in Ca^{2+} transients along the dendrites (Margrie et al., 2001).

Evoked neuronal activity can also produce a long lasting modulation of Ca^{2+} transients. Local applications of glutamate to the basal dendrites of neurons in the prefrontal cortex result in Ca^{2+} plateaus that persist beyond the duration of dendritic spikes. These plateaus are spatially restricted to the input site and are governed by NMDA-mediated small amplitude depolarisation (Milojkovic et al., 2007). The persistence of Ca^{2+} transients may have important consequences for the integration of spatially or temporally neighbouring synaptic inputs. Inhibitory input, on the other hand, can block Ca^{2+} influx into the cell and selectively prevent the induction of Ca^{2+} spikes (Tsubokawa and Ross, 1996; Larkum et al., 1999b; Larkum et al., 2007). In CA1 pyramidal neurons, supralinear synaptic input inhibits subsequent dendritic spikes in that specific input region due to the slow inactivation of Na^+ channels. If this supralinear synaptic input is strong enough to evoke a somatic AP, then the resulting bAP attenuates subsequent dendritic spikes in all dendritic branches (Remy et al., 2009).

Dendritic Excitability and Sensory Processing

Active dendritic mechanisms are recruited during the normal processing of sensory information as well as following sensory deprivation. For example, *in vivo* recordings from anaesthetised rats performed in the distal dendrites of neocortical pyramidal neurons in the barrel cortex show that whisker stimulation results in complex spikes coupled with large Ca^{2+}

transients. These complex spikes arising from local EPSPs caused by whisker deflections are large and slow depolarisations with superimposed bursts of Na^+ APs (Helmchen et al., 1999). The spiny stellate neurons of L4 also play an active role in shaping the responses to whisker sensing through the generation of NMDA spikes (Lavzin et al., 2012). Sensory deprivation, on the other hand, caused by whisker trimming, results in a significant lowering of the critical frequency threshold necessary for the generation of dendritic Ca^{2+} spikes. This is most likely mediated by a reduction in h channel density, which occurs as a consequence of sensory deprivation (Breton and Stuart, 2009).

Dendritic spikes can also mediate the integration of inputs from spatially separated and functionally distinct neuronal pathways, provided that their co-activation is temporally synchronous. For example, in an object-location task mice are trained to lick a water port for a reward when a certain object is positioned in the 'correct' location. The mice use their whiskers to sense in which location the object is positioned at a given time and they have to withhold licking if the object is in the 'wrong' location. During whisker touches at particular object locations, large-amplitude, global Ca^{2+} signals occur throughout the apical tuft dendrites of L5 pyramidal neurons in the barrel cortex. These are a result of pairing the vibrissal sensory input with the activity of the primary motor cortex (Xu et al., 2012). The threshold and duration of this dendritic electrogenesis, and the resulting integration of information from different dendritic compartments is controlled by K^+ channels (Harnett et al., 2013).

Dendritic Excitability and Learning

One of the first accounts of learning induced plasticity of dendritic excitability in mammals comes from intracellular dendritic recordings performed in cerebellar Purkinje cells of rabbits that underwent delay eyelid conditioning. In this learning task rabbits are taught to associate a tone (conditioned stimulus) with either an air puff or a weak periorbital electrical stimulation (unconditioned stimulus). In brain slices from animals that underwent this form of associative conditioning, the threshold for evoking a dendritic Ca^{2+} spike was reduced when compared to controls. Interestingly, the lower threshold for dendritic spike initiation was still present one month after training (Schreurs et al., 1997; 1998).

Dendritic excitability is also likely to play an important role during exploratory learning. By using specific input patterns designed to match those occurring during exploratory sharp waves (SPW; O'Neill et al., 2006) and a transient application of carbochol to imitate the neuromodulatory state of exploratory behaviour (Hasselmo and Giocomo, 2006) it is possible to mimic an exploratory state in acute hippocampal slices (Losonczy et al., 2008). A protocol providing such spatially clustered and temporally synchronous input results in increasing the coupling between two proximal dendritic branches in a process called branch strength potentiation (BSP). BSP means that after stimulation a weak terminal daughter branch that originates from a strong parent dendrite becomes strengthened. These daughter branches evoke stronger and larger dendritic spikes with an enhanced probability of spreading into the strong parent branch. This form of plasticity is branch specific as it is restricted only to the specific branches stimulated. Similar results can be obtained by pairing local dendritic spikes with bAPs using an associative theta-pairing protocol (Losonczy et al., 2008). Behaviourally, when an animal is exposed to an enriched environment, there is an increase in the dendritic

spike strength in daughter branches coupled to strong parent dendrites closely resembling the pattern of BSP (Makara et al., 2009). This compartmentalised form of dendritic plasticity is most likely caused by a downregulation of dendritic $K_v4.2$ channels (Losonczy et al., 2008; Makara et al., 2009).

The activity of voltage-dependent A-type K^+ channels is indeed required for normal behavioural learning. $K_v4.2$ knockout mice have been shown to have spatial learning impairments in the Morris water maze task (Lockridge and Yuan, 2011) as well as in contextual fear conditioning (Lugo et al., 2012). In contrast, HCN1 channels were shown to constrain spatial learning and memory. Upon the forebrain-restricted deletion of the *HCN1* gene, mice show an improvement in spatial learning in the Morris water maze task as well as an enhanced short and long-term memory of the platform location. This could partially be explained by the fact that *HCN1* deletion results in an enhancement in theta frequency oscillations, which are thought to be important for spatial memory encoding and storage (Nolan et al., 2004). Interestingly, however, HCN1 channel activity is critical for the normal learning of motor coordination due to its role in mediating synaptic input integration by cerebellar Purkinje neurons (Nolan et al., 2003).

Dendritic Excitability and Synaptic Plasticity

LTP and LTD are the most studied cellular correlates of learning and memory. LTP and LTD can be defined as a long-lasting and persistent increase or decrease, respectively, in synaptic efficacy (Bliss and Lømo, 1973; Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). We now know that dendritic excitability can induce or be induced by LTP (Johnston et al., 2003). Metaplasticity is a phenomenon whereby synaptic and cellular activity can induce neuronal changes, which in turn alter the neuron's ability to undergo subsequent synaptic plasticity (Abraham and Bear, 1996). It has therefore been suggested that dendritic plasticity could act as a substrate for metaplasticity (Frick and Johnston, 2005; Chen et al., 2006).

LTP induction is accompanied by a persistent and local NMDA receptor-dependent increase in dendritic excitability. This plasticity is manifested by an augmentation of the bAPs amplitude and an accompanying boost in Ca^{2+} signals. More importantly, the increase in dendritic excitability favours the backpropagation of APs into the potentiated dendritic region (Frick et al., 2004). This enhancement in excitability is mediated by a hyperpolarised shift in the inactivation curve of A-type K^+ channels resulting in a reduction of the transient A-type K^+ current allowing the AP to backpropagate more efficiently (Watanabe et al., 2002; Frick et al., 2004). This change in dendritic excitability is local and persistent and depends on MAPK activation. When MAPK is blocked, LTP can still be induced by strong stimulation but the associated change in dendritic excitability is no longer present (Rosenkranz et al., 2009). The activation of MAPK is linked to PKA activity. In CA1 neurons PKA acts on MAPK to modulate A-type K^+ currents and bAP amplitude (Yuan et al., 2002) and is necessary for LTP induction (Huang and Kandel, 1994; Nguyen and Kandel, 1996).

When bAPs are paired with local subthreshold EPSPs in a theta burst stimulation protocol the result is a boost of the dendritic AP amplitude, an increased Ca^{2+} influx and a consequent induction of NMDA receptor-dependent LTP (Magee and Johnston, 1997; Frick et al., 2004; Sjöström and Häusser, 2006). Most likely, this process is mediated by the inactivation of A-type K^+ channels resulting in an increased efficacy of AP backpropagation.

The enhancement in AP backpropagation then provides the necessary depolarisation for the release of the voltage-dependent Mg^{2+} block of NMDA receptors and the subsequent induction of LTP (Watanabe et al., 2002). More importantly, LTP does not occur when bAPs are blocked by a local application of TTX to the proximal dendritic region. Even though synaptic potentials and somatic APs can still be evoked, LTP is no longer induced (Magee and Johnston, 1997).

BAPs can act as a bidirectional switch between LTP and LTD induction causing the sign of the potentiation to be dependent upon the precise timing of bAP and/or the location of the activated synapses (Letzkus et al., 2006; Sjöström and Häusser, 2006). Using the same potentiation protocol Sjöström and Häusser (2006) induced LTP at proximal synapses and LTD at more distal synapses of neocortical pyramidal neurons. This distance-dependent switch in the synaptic potentiation sign is due to the unreliable propagation of APs into distal dendritic branches. LTD could be switched to LTP at distal synapses by a local depolarisation of the dendritic membrane during the potentiation protocol. The pairing of bAPs with local membrane depolarisations resulted in supralinear Ca^{2+} signals, which were then able to boost the otherwise-decaying bAP and result in LTP induction (Sjöström and Häusser, 2006).

BAPs are not necessary for the induction of LTP under all conditions. It is possible that when AP propagation is blocked by TTX application and/or hyperpolarisation, dendritic spikes can provide the Ca^{2+} influx and depolarisation necessary to induce LTP (Golding et al., 2002). Dendritic spikes also seem to be critical for LTP induction when APs do backpropagate. The precise pairing of high frequency somatic AP bursts with EPSPs results in supralinear increases in Ca^{2+} signals that leads to the activation of NMDA receptors (Kampa and Stuart, 2006).

The induction of LTP and LTD is also accompanied by changes in dendritic excitability linked to the up or downregulation of h channel activity (Wang et al., 2003; Fan et al., 2005; Brager and Johnston, 2007). This bi-directional plasticity and the associated changes in h channel function have important consequences for the summation of synaptic potentials. Additionally, LTP induction has been shown to alter the resonance frequency of neurons also through the modulation of h channels (Narayanan and Johnston, 2007, 2008). This has important consequences for membrane potential oscillations, which are thought to be involved in memory encoding and network synchronisation (Buzsáki, 2002; Engel et al., 2001).

EPSP-Spike Potentiation

The induction of LTP results in a persistent potentiation of synaptic responses (Bliss and Lømo, 1973). However, LTP in the hippocampus also results in an enhanced probability that the postsynaptic neurons will fire APs to a given excitatory input (population spike). This phenomenon is termed EPSP-spike potentiation (E-S potentiation, Bliss et al., 1973). E-S potentiation is strongly mediated by inhibitory synaptic transmission and it has been suggested that E-S potentiation is a result of an alteration in the balance between excitation and inhibition following LTP induction (Abraham et al., 1987; Chavez-Noriega et al., 1989). However, it is known that hippocampal E-S potentiation can be induced in the absence of inhibition (Asztely and Gustafsson, 1994; Jester et al., 1995; Daoudal et al., 2002). This suggests that an additional component might be necessary for E-S potentiation, namely

intrinsic changes in excitability (Taube and Schwartzkroin, 1988; Daoudal et al., 2002). One piece of supporting evidence comes from Wang et al. (2003), who showed that LTP induction in CA1 pyramidal neurons causes a supralinear increase in EPSP summation. This increase is mediated by a downregulation of dendritic h channels (Wang et al., 2003), which are known to reduce the temporal summation of synaptic inputs (Magee, 2000; Poolos et al., 2002). LTD on the other hand reduces the linearity of spatial EPSP summation. It is known that the supralinearity of spatial and temporal synaptic input summation can shape the spiking activity of the neuron (Magee, 1999; 2000). Indeed, Xu et al., (2005) found that LTP induction increased intrinsic excitability. This was manifested by a progressive decrease in AP threshold that could serve as a mechanism for E-S potentiation. This change was definitely due to intrinsic properties because it occurred in the presence of synaptic activity blockers as well as $GABA_A$ and $GABA_B$ blockers and is likely to be mediated by a hyperpolarised shift in the activation curve of voltage-gated Na^+ channels (Xu et al., 2005).

In contrast to Wang et al. (2003) and Xu et al. (2005), Fan et al. (2005) showed that LTP induction resulted in a global decrease in neuronal excitability. This was marked by an increase in AP threshold and more somatically injected current necessary to evoke the same number of APs as before LTP induction. These changes were mediated by an upregulation of h channels (Fan et al., 2005). These seemingly conflicting results have recently been reconciled by Campanac et al., (2008), who showed that different LTP induction protocols could affect h conductance differently. A strong theta-burst protocol results in a homeostatic upregulation of I_h conductance, whereas more moderate levels of LTP induced through STDP protocols trigger E-S potentiation through the downregulation of I_h conductance (Campanac et al., 2008).

In conclusion, the wide variety of membrane voltage-gated ion channels endows dendrites with their active properties that are critical for neuronal information processing and plasticity. Most excitatory synapses are made onto dendrites and it is the dendrites then that govern the manner and efficacy with which synaptic input is integrated and propagated into the rest of the neuron. This means that any alteration in signal processing that occurs at the level of the dendrites will critically alter the output message of the neuron. This endows dendrites with significant computational power and thus makes them important targets for neuronal plasticity research.

Pathological Changes in Dendritic Excitability

Alterations in the function and/or expression levels of dendritic voltage-gated ion channels may be implicated in a number of CNS disorders (Table 4). These so-called channelopathies can be inherited or acquired, or sometimes results a mix of both in some groups of disorders, as illustrated below for epilepsy related dysfunctions (Beck and Yaari, 2008; Mantegazza et al., 2010; Poolos and Johnston, 2012).

Table 4. Examples of ion channels involved in CNS disorders

CNS disease	Ion channels	Structure	Changes in dendritic excitability	Ref.
Alzheimer's disease	$K_v4.2$ channels			
	Decreased/blocked A-type current	*Hippocampus*	- Increased bAP amplitude	*Good and Murphy, 1996; Xu* et al., *1998; Chen* et al., *2005; Morse* et al., *2010*
	Increased A-type current	*Cerebellum*	- Decreased excitability?	*Ramsden* et al., *2001; Plant* et al., *2006*
	$Na_v1.1$ channels			
	Decreased expression / Decreased somatic excitability	*Cortical interneurons*	- Decreased excitability?	*Verret* et al., *2012*
Autism Spectrum Disorders	BK channels			
	Disruption of the KCNMA1 gene	*Genome studies*	- Altered dendritic excitability?	*Laumonnier* et al., *2006*
	$K_v4.1$ channels			
	Mutations in the KCNJ10 gene	*Genome studies*	- Altered dendritic excitability?	*Sicca* et al., *2011*
	L-type Ca^{2+} channels			
	Deletion of the CACNA1C gene	*Genome studies*	- Altered dendritic excitability?	*Smith* et al., *2012*
	Deletion of the CACNA2D4 gene	*Genome studies*	- Altered dendritic excitability?	
	T-type Ca^{2+} channels			
	Mutations in the CACNA1H gene	*Genome studies*	- Altered dendritic excitability?	*Splawski* et al., *2006*

Table 4. (Continued)

CNS disease	Ion channels	Structure	Changes in dendritic excitability	Ref.
Bipolar Disorders	L-type Ca^{2+} channels *Genetic variation in CACNA1C* M-type K^+ channels *Altered expression of KCNQ2, KCNQ3*	*Genome studies* *Post-mortem studies*	- Altered dendritic excitability? - Increased excitability?	*Bhat* et al., *2012* *For review, Judy* et al., *2013*
Down Syndrome	$K_{ir}3.2$ and $K_{ir}4.2$ channels *Located on the DCR1 region of the chromosome 21*	*Genome studies*	- Increased excitability?	*Ohira* et al., *1997; Gosset* et al., *1997*
EAST Syndrome	$K_{ir}4.1$ channels *Mutated channels showed altered function*	*Overexpression in HEK cells*	- Increased excitability?	*Reichold* et al., *2010*
Episodic Ataxia	$K_v1.1$ channels *Mutated channels showed smaller currents and altered gating properties*	- Overexpression in Xenopus Oocytes	- Increased excitability?	*Adelman* et al., *1995; Zerr* et al., *1998*
Epilepsy Inherited epilepsy	$Na_v1.1$ channels *Loss of function mutations* $Na_v1.2$ channels *Impaired channel function*	*Hippocampus and Cortex* *Neocortex primary cultures*	- Decreased AP firing in GABAergic neurons - Decreased excitability?	*Reviewed in Catterall* et al., *2010* *Scalmani* et al., *2006; Misra* et al., *2008*

CNS disease	Ion channels	Structure	Changes in dendritic excitability	Ref.
Acquired epilepsy	$K_v7.2/7.3$ channels (M-type K^+ current) *Decreased stability, altered gating* *Increased excitability*	*Hippocampus*	- Increased dendritic excitability due to increased AP firing rate?	*Reviewed in Miceli* et al., *2011.* *Soldovieri* et al., *2006;* *Soldovieri* et al., *2007* *Peters* et al., *2005*
	T-type Ca^{2+} channels *Mutated channels showed altered gating properties*	*Overexpression in HEK cells*	- Altered dendritic integration?	*Khosravani* et al., *2004*
	BK channels *Mutated channels showed gain of function*	*Overexpression in Xenopus oocytes*	- Altered neuronal and dendritic excitability?	*Du* et al., *2005*
	A-type K^+ channels *Decreased $K_v4.2$ expression and A-type current*	*Hippocampus*	- Increased bAP amplitude	*Bernard* et al., *2004;* *Chen* et al., *2006; Lugo* et al., *2008*
	Na^+ channels *Increased Na^+ currents*	*Hippocampus*	- Increased neuronal bursting → Increased bAP/ Altered STDP?	*Chen* et al., *2011*
	H channels *Decreased HCN1 activity*	*Hippocampus and entorhinal cortex*	- Increased dendritic excitability /Decreased dendritic resonance	*Shah* et al., *2004; Jung* et al., *2007; Marcelin* et al., *2009; Jung* et al., *2011*
	T-type Ca^{2+} channels *Increased T-type currents*	*Hippocampus*	- Increased intrinsic burst firing → Increased bAP/ altered STDP?	*Becker* et al., *2008*

Table 4. (Continued)

CNS disease	Ion channels	Structure	Changes in dendritic excitability	Ref.
	BK channels *Gain of function* *Downregulation, reduced expression*	*Neocortex* *Hippocampus (CA3)*	- Increased firing - Increased excitability?	*Shruti* et al., *2008* *Pacheco-Otalora* et al., *2008*
	SK channels *Functional downregulation*	*Hippocampus*	- Increased dendritic excitability	*Cai* et al., *2007*
Fragile X Syndrome	L-type Ca^{2+} channels *Decreased expression of $Ca_v1.3$, altered Ca^{2+} signaling*	*Frontal/Prefrontal cortex*	- Decreased dendritic Ca^{2+} signaling/ Altered STDP	*Chen* et al., *2003;* *Meredith* et al., *2007*
	H channels *Increased HCN1 expression*	*Hippocampus*	- Reduced EPSP temporal summation/Reduced excitability?	*Brager* et al., *2012*
	$K_v4.2$ channels *Decreased $K_v4.2$ expression* *Increased $K_v4.2$ expression*	*Hippocampus* *Hippocampus*	- Altered dendritic excitability/ dendritic function?	*Gross* et al., *2011* *Young-Lee* et al., *2011*
	$K_v3.1b$ channels *Decreased $K_v3.1b$ immunoreactivity and current*	*Brainstem*	- Altered firing rate → Altered bAP / STDP?	*Strumbos* et al., *2010*
	BK channels *Decreased BKCa1.1 expression*	*Neocortex*	- Altered neuronal firing/ dendritic excitability?	*Liao* et al., *2008*

CNS disease	Ion channels	Structure	Changes in dendritic excitability	Ref.
Parkinson's Disease	H channels *Reduced h channels expression and h current*	*External Globus Pallidus*	-Altered dendritic function/ Reduced resonance?	*Chan* et al., *2011*
Schizophrenia	L-type Ca^{2+} channels *Genetic variation in CACNA1C*	*Genome studies*	- Altered dendritic excitability/ function?	*Bhat* et al., *2012*
	BK channels *Decreased expression of BK mRNA*	*Post-mortem studies, Prefrontal cortex*	- Altered neuronal firing/ dendritic excitability?	*Zhang* et al., *2006*
Timothy Syndrome	L-type Ca^{2+} channels *Mutated channels showed altered gating properties*	*Overexpression in HEK cells*	- Altered dendritic function/ Reduced dendritic spikes?	*Barrett and Tsien, 2008*

The relation of a channelopathy to the etiology of a CNS disorder is often difficult to assess. Indeed, ion channel dysfunctions can contribute to pathogenesis but can also be the consequence of a pathological state. For example, specific mutations in the $Ca_V1.2$ subunit of L-type Ca^{2+} channels are the cause for Timothy syndrome (Splawski et al., 2004), whereas in Alzheimer's disease the presence of Aß peptides aggregates consequently modulate $K_V4.2$ subunits of A-type K^+ channels (Chen, 2005; Dong et al., 2012). In the following section we will describe examples of channelopathies in some neurodevelopmental, neuropsychiatric, neurodegenerative, and acquired CNS disorders. We will summarise established links between channelopathies and dendritic pathophysiology in these disorders, or attempt to make this link where it seems likely.

Neurodevelopmental Disorders

Several voltage-gated ion channel dysfunctions have been linked to neurodevelopmental disorders, in particular to Autism Spectrum Disorders (ASD) and related syndromes such as Fragile X Syndrome (FXS) and Timothy Syndrome (TS). Most of the molecular advances in our understanding of the pathophysiology of these disorders stem from studies using suitable mouse models of genetic syndromes associated with ASD, such as FXS, Timothy's Syndrome, and Rett Syndrome (Hampson et al., 2012).

Autism Spectrum Disorders (ASD)

ASD affect more than 1% of the population and are characterised by impaired social interaction, communication difficulties and stereotyped behaviours (Baio, 2012). However, the etiology of ASD remains largely unknown despite the identification of several genetic and environmental risk factors (Benvenuto et al., 2009). Many genes have been associated with the development of ASD, including genes encoding proteins important for the formation and plasticity of synapses (Benvenuto et al., 2009; Smith and Sadee, 2011), as well as voltage-gated Ca^{2+} channels (Krey and Dolmetsch, 2007). Amongst the voltage-gated Ca^{2+} channels, mutations in subunits for T-type and L-type Ca^{2+} channels have been repeatedly identified in patients diagnosed with ASD (Splawski et al., 2006; Smith et al., 2012). T-type Ca^{2+} channels are expressed both in the shaft and spines of dendrites in CA1 pyramidal neurons (Christie et al., 1995; Sabatini and Svoboda, 2000), and in dendritic spines of neocortical pyramidal neurons (Koester and Sakmann, 2000). In CA1 pyramidal neurons, low-threshold activated T-type Ca^{2+} channels open following subthreshold depolarisation during a train of EPSPs, producing a local increase in internal Ca^{2+} concentration, which could participate in local dendritic integration (Magee et al., 1995). These channels also generate low-threshold spikes that lead to burst firing and intracellular Ca^{2+} oscillations (Chevalier et al., 2006). Ca^{2+} influx through T-type Ca^{2+} channels may help to stabilise synapses, contribute to the persistence of LTP, and be necessary for retrieval of context-associated memory (Magee et al., 1995; Chen et al., 2012). L-type Ca^{2+} channels are present in the dendritic shaft of CA1 pyramidal neurons (Christie et al., 1995), and in both dendrites and spines in neocortical pyramidal neurons (Markram et al., 1995; Koester and Sakmann, 1998). In CA1 and L5 pyramidal neurons, these channels contribute to back-propagating AP and to the generation of dendritic spikes (Frick et al., 2003; Almog and Korngreen, 2009; Grewe et al., 2010; Perez-Garci et al., 2013). Furthermore, L-type Ca^{2+} channels contribute to the induction of LTP in

hippocampal/neocortical pyramidal neurons (Bi and Pool, 1998; Meredith et al., 2007). Thus, modification of any of these Ca^{2+} channels in pathology is likely to alter dendritic excitability, as it has for example been described in a mouse model of FXS (Meredith et al., 2007; see below).

Fragile X Syndrome (FXS)

FXS is the most frequently inherited form of intellectual disability and the most well characterised cause of ASD, with about one third of FXS patients also diagnosed with ASD (Gallagher and Hallahan, 2012). FXS is due to a transcriptional silencing of the *Fmr1* gene, which encodes the FMRP protein (Pieretti et al., 1991). Several ion channels that are important regulators of dendritic function are altered in *Fmr1* knockout (*Fmr1*KO) mice (the mouse model for FXS). These ion channels include L-type Ca^{2+} channels, voltage-gated K+ channels and h channels (Chen et al., 2003; Meredith et al., 2007; Gross et al., 2011; Lee et al., 2011; Strumbos et al., 2010; Liao et al., 2008; Brager et al., 2012).

L-type Ca^{2+} channel expression levels are decreased in the prefrontal cortex (Chen et al., 2003; Meredith et al., 2007). The functional consequences of this downregulation are an altered Ca^{2+} signalling and a higher threshold for STDP, a form of plasticity relying on dendritic excitability (Meredith et al., 2007; see section Dendritic excitability and synaptic plasticity).

Several channels whose expression is altered in *Fmr1*KO mice fall into the class of K^+ channels. Two recent studies present conflicting viewpoints with respect to changes in $K_v4.2$ (Gross et al., 2011; Lee et al., 2011), the major subunit of A-type K^+ channels, highlighting its possible alteration in FXS. Gross et al. (2011) observed reduced expression levels of $K_v4.2$ subunit in both hippocampus slices and cortical lysates of *Fmr1*KO mice, and that FMRP positively regulated $K_v4.2$ mRNA translation. Given the known role of these channels in the physiology of hippocampal/neocortical neurons (Hoffman et al., 1997; Kim et al., 2005; Burkhalter et al., 2006; Carrasquillo et al., 2012), a decrease in A-type K^+ channels is expected to increase dendritic excitability and could therefore contribute to the prevalence of epileptic seizures among FXS patients (Hagerman et al., 2009). In contrast, Lee et al., (2011) found that FMRP regulates dendritic targeting, and suppresses the translation of $K_v4.2$ mRNA in hippocampal primary cultures under basal conditions, and that this suppression can be relieved following NMDAR activation. The different conclusions of these studies could be explained by differences in the techniques used, in the genetic background of the *Fmr1*KO mice, or in the age of the animals (Gross et al., 2011; Lee et al., 2011), and more investigations are thus still required to rule on the expression state of $K_v4.2$ in FXS.

The mRNA for $K_v3.1$ channels, that produce a fast delayed rectifyer current, has also been identified (Darnell et al., 2001) and validated (Strumbos et al., 2010) as a binding target for FMRP. In the brain stem of *Fmr1*KO mice, Strumbos et al. (2010) showed a reduction in expression of $K_v3.1$ in response to acoustic stimulation, associated with a decrease in TEA-sensitive K^+ current. Axonal $K_v3.1$ is known to regulate the neuronal spiking frequency (Gu et al., 2012), and could therefore indirectly affect dendritic excitability through the regulation of the frequency of bAPs (see section Dendritic spikes). In addition, a splice variant of $K_v3.1$ is targeted to the dendrites in hippocampal and L5 pyramidal neurons and could therefore play a direct role at this location (Xu et al., 2007; Hay et al., 2011; Gu et al., 2012).

BK channels — big conductance Ca^{2+}- and voltage-activated K^+ channels — might also be altered in FXS. Indeed, western blots from cortical lysates of *Fmr1*KO mice revealed a

decreased expression of the pore forming α-subunit of BK channels, KCNMA1α (Liao et al., 2008). Moreover, a recent study showed that FMRP could directly bind the BK_{Ca} channel β4 accessory subunit, thereby regulating their Ca^{2+} sensitivity (Deng et al., 2013). BK channels are important regulators of dendritic excitability controlling AP back-propagation and dendritic spike threshold (Benhassine and Berger, 2009). BK channels also play a key role in controlling AP firing frequency (Ly et al., 2011), and would hence also indirectly regulate dendritic function by determining bAP firing pattern. Dysfunction of these channels in FXS could therefore greatly alter both somatic and dendritic activity. For example, their downregulation would be expected to alter somatic AP firing, increase AP backpropagation, and reduce the threshold for dendritic spikes (Shruti et al., 2008; Benhassine and Berger, 2009).

Finally, changes in hyperpolarisation-activated cation channels, or h channels, expression have also been reported in *Fmr1*KO mice (Brager et al., 2012). H channels provide a brake in dendritic excitability and dendritic electrogenesis (Williams and Stuart, 2000b; Breton and Stuart, 2009). In the mouse model of FXS, Brager et al., (2012) found an increase in HCN1 subunit expression in the CA1 area of the hippocampus, resulting in an altered h channel-dependent form of plasticity in CA1 pyramidal neurons.

Altogether, these studies demonstrate dysfunctions of Ca^{2+}, K^+, BK and h channel strongly suggest that dendritic processing is altered in FXS.

Timothy Syndrome (TS)

TS is a rare disorder characterised by physical and neurological problems expressed primarily as cardiac abnormalities and ASD (Splawski et al., 2004). The symptoms of TS are caused by an ion channel dysfunction, namely mutations in the *Cacna1c* gene encoding the $Ca_v1.2$ subunit of the L-type Ca^{2+} channels (Splawski et al., 2004). Expression of the most common mutated form of the gene in HEK cells showed defects in the voltage- and Ca^{2+}-dependent inactivation of the resulting L-type Ca^{2+} channel (Barret and Tsien, 2008). Furthermore, the authors showed that the TS mutation greatly slowed the voltage dependent inactivation of L-type Ca^{2+} channels, at the same time increasing the kinetics of the Ca^{2+} -dependent inactivation. These alterations in the gating properties are likely to lead to alterations in Ca^{2+} signalling in dendrites, thereby affecting intrinsic excitability. A decrease in Ca^{2+} signalling could, for example, impair dendritic excitability by reducing the occurrence and amplitude of dendritic spikes and decreasing the amplitude of bAP. It could also affect plasticity processes such as STDP (Meredith et al., 2007).

Neuropsychiatric Disorders

Neuropsychiatric disorders, such as schizophrenia and bipolar disorders, are complex pathologies, with complicated diagnosis due to their multifactorial symptoms and intricate etiology (Sullivan et al., 2012). Dysfunctions in both Ca^{2+} and K^+ channels, two major classes of ion channels determining cellular excitability, have been associated with these disorders.

Genetic variations in the pore-forming α subunit of L-type Ca^{2+} channels, $Ca_v1.2$, were found in patients with schizophrenia and bipolar disorders (for review, Bhat et al., 2012). As described previously, L-type Ca^{2+} channels are particularly important for dendritic Ca^{2+}

signaling and dendritic electrogenesis in neocortical pyramidal neurons (Almog and Korngreen, 2009; Grewe et al., 2010; Perez-Garci et al., 2013). This is crucial because the neocortex is a pivotal brain structure involved in many cognitive processes such as consciousness, attention, decision-making and sensory perception, some of which are altered in these two disorders (Arguello and Gogos, 2012).

Bipolar disorders have also been associated with mutations in KCNQ2 and KCNQ3, the genes encoding two K^+ channels subunits underlying M-type current (for review, Judy and Zandi, 2013). Although mainly expressed at the somatic and axonal level, M-type channels not only control AP initiation and neuronal excitability, but can also regulate E-S coupling (Brown and Passmore, 2009; Shah et al., 2011). Their alterations could therefore also affect dendritic information processing.

In addition, evidence from the literature suggests that BK channels might also be dysregulated in schizophrenia. Studies showed that neuroleptics can modulate BK channel function, indicating that BK channel openers could be used as alternative pharmacological treatment for schizophrenia (Dinan, 1987; Akhondzadeh et al., 2002). More recently, a post-mortem study from patients diagnosed with schizophrenia showed a reduction in BK channel mRNA in the prefrontal cortex of these subjects, compared to controls (Zhang et al., 2006). A reduction in BK channels could result in an altered neuronal firing pattern, and increased dendritic excitability (see section FXS). Together, these findings argue for a role of BK channel dysfunction in the etiology of schizophrenia.

Neurodegenerative Disorders

Neurodegenerative disorders are characterised by a progressive neuronal dysfunction, often associated with the loss of a neuronal population. The most common neurodegenerative diseases are Alzheimer's disease (AD) and Parkinson's disease (PD), both of which have been associated with an alteration in the ion channels involved in neuronal excitability.

Alzheimer's Disease (AD)

AD is a progressive and irreversible brain disease leading to dementia and memory loss (Small et al., 2001). The accumulation of the amyloid β (Aβ) peptide, leading to the formation of so-called amyloid plaques, is a key marker of the disease, and is believed to contribute to excitotoxicity and neuronal degeneration (Gandy, 2005). However, how Aβ mediates excitotoxicity remains unclear. One mechanism that has been proposed is through the modulation of $K_v4.2$ channels. The first evidence supporting this hypothesis was shown *in vitro* by Good and Murphy (1996). Using hippocampus primary cultures, they demnstrated that application of synthetic Aβ to neurons lead to a reduction in A-type K+ current, thereby increasing neuronal excitability. An independent study, using cerebellar granule and cortical cell cultures, suggested that Aβ effect on A-type K+ current is cell-type specific and depends on the aggregation state of the peptide (Ramsden et al., 2001). More recently, Chen (2005) reported that Aβ also reduces A-type K^+ current in a hippocampal slice preparation. More specifically, they showed that Aβ inhibits dendritic A-type K+ current, resulting in an increase in back-propagating AP amplitude in the apical dendrite and in the associated Ca^{2+} influx. Aβ could also regulate A-type K^+ current in CA1 pyramidal neuron oblique dendrites,

as suggested by a computational study (Morse et al., 2010). Indeed, using a computer model the authors showed that A-type K^+ channels would be particularly sensitive to Aβ modulation in these thin dendrites, and that therefore Aβ accumulation would greatly affect excitability at this location. Moreover, a decrease in A-type K+ current could contribute to the hyperexciability phenotype found in hippocampal neurons in a mouse model of AD (Busche et al., 2012). In summary, A-type K^+ channels play a major role in regulating dendritic excitability in CA1 pyramidal cells, and alteration of their function by Aβ interaction is therefore likely contribute to excitotoxity and eventually to neuronal loss in AD.

More recently, a role for $Na_v1.1$ channels was also found in the pathophysiology of AD (Verret et al., 2012). In this study, the authors showed a decrease in $Na_v1.1$ subunit expression in parvalbumin-positive interneurons of the parietal cortex of both AD patients and a mouse model of AD. In the mouse model of AD, this finding was correlated with impairment in inhibitory connections in the parietal cortex and network hypersynchrony (Verret et al., 2012). Together, their results suggest that a decreased expression of Na^+ channels in the cortex could contribute to the epileptiform activity and seizures observed in AD patients (Verret et al., 2012; Vossel et al., 2013). Although $Na_v1.1$ channels are predominantly expressed in the axon initial segment in several brain regions (including cerebral cortex and hippocampus) thereby controlling AP initiation and propagation (Duflock et al., 2008), they are also expressed in the somatodendritic compartment of L5 and CA1 pyramidal neurons (Gong et al., 1999) where changes in their expression would alter dendritic excitability.

Parkinson's Disease (PD)

PD is a debilitating neurodegenerative disorder characterised by severe motor symptoms (Lotharius and Brundin, 2002). It is associated with a loss of dopaminergic neurons in the substantia nigra pars compacta, which results primarily in the depletion of dopamine in the striatum (Lotharius and Brundin, 2002), a major sub-cortical structure controlling voluntary movement. However, another key marker of the disease is the altered activity of the external globus pallidus (GPe), a distinct basal ganglia nucleus crucial for movement control. In PD the GPe activity becomes rhythmic and synchronous, especially at late-stage of the disease (Hammond et al., 2007). Recently, this synchronisation of GPe activity has been associated with h channel channelopathy (Chan et al., 2011). In their study, the authors showed that depletion in dopamine leads to a decrease in h current in GPe neurons through a downregulation of the principal (HCN1-4) and accessory (TRIP8b) subunits underlying to I_h. Moreover, they demonstrated that overexpressing HCN2 subunit *in vivo* in a model of PD restored normal activity to the GPe. At the dendritic level, a downregulation of h channels could affect EPSP summation, backpropagation efficacy and the threshold for dendritic spikes (Kole et al., 2006; 2007). It could notably cause an increase in excitability and Ca^{2+} electrogenesis, as it has been shown in the context of sensory deprivation (Breton and Stuart, 2009). This is so far the only example showing that a channelopathy, induced by dopamine loss, could contribute to the pathophysiology of PD.

Acquired Brain Disorders

Acquired brain disorders are defined as alterations in brain function that are not congenital or caused by birth trauma and that are attributable to external trauma. This external trauma can in some cases trigger alterations in ion channels function as it has been shown in some forms of acquired epilepsy. Indeed, channelopathies are not only associated with genetically determined human epilepsies (for review Mantegazza et al., 2010; D'Adamo et al., 2013), but can also be a consequence of brain trauma-induced epilepsies (for review Poolos and Johnston, 2012).

In animal models of acquired temporal lobe epilepsy (TLE), there is a decrease in dendritic A-type current, associated with a down-regulation of $K_v4.2$ expression, and an increase dendritic excitability in the hippocampus (Bernard et al., 2004; Su et al., 2008). Moreover, post-mortem studies in tissue from patients with hippocampal sclerosis, a common feature of TLE, showed a decreased expression of $K_v4.2$ in hippocampal dendrites (Aronica et al., 2009). As described above, A-type K^+ current regulates AP backpropagation and amplitude of EPSPs, and increases the threshold for dendritic spikes (Hoffman et al., 1997, Ramakers et al., 2002). Together, these findings argue for a major role of $K_v4.2$ dowregulation in epileptogenesis resulting in an overall increase in dendritic excitability. Another K^+ current altered in aquired epilepsy is SK current (small conductance Ca^+-activated K^+ channel-mediated hyperpolarizing outward current). A reduction in SK current was, for instance, found in CA1 pyramidal neurons in a chronic TLE model (Schulz et al., 2012). In hippocampal pyramidal dendrites, SK channels limit the duration of plateau potential (Cai et al., 2004). A decrease in this channel function could therefore alter dendritic integration and result in an increased dendritic excitability.

Epileptic seizures can also modulate another key determinant of dendritic excitability: h channels (Chen et al., 2001; Shah et al., 2004). Using a rat model of TLE, the authors showed that seizures induce a diminution in h currents in the entorhinal cortex, due to a downregulation of HCN1 and HCN2 subunits, associated with an increased excitability. In another model of epilepsy, a shift in h-current activation curve has been shown in the hippocampus following hypothermia-induced seizures (Chen et al., 2001). This modification of h channels kinetics resulted in a neuronal hyperexcitability reflected by an increased post-inhibitory rebound firing (Chen et al., 2001). Based on these findings and on the known role of I_h in regulating neuronal excitability, h channels may represent new targets for seizure control (Chen et al., 2002). Moreover, following developmental seizures there is a decrease in HCN1 expression in CA1 (Brewster et al., 2002), region where HCN1 is also expressed in parvalbumin-expressing interneurons (Lörincz et al., 2002; Brewster et al., 2002). Given the fundamental role of inhibition in controlling dendritic excitability (Müller et al., 2012), altered interneurons activity due to HCN loss could lead to an increased dendritic excitability in their pyramidal cell targets.

Lastly, Ca^{2+} channels function is also likely to be altered in acquired epilepsy. A study in CA1 pyramidal cells indeed revealed a significant up-regulation of a T-type Ca^{2+} channels associated with elevated burst firing of these neurons after a single episode of status epilepticus (Su et al., 2002, reviewed in Yaari et al., 2007).

Together, these examples emphasise the role of channelopathies in the pathophysiology of epileptic syndromes.

Conclusion

While the importance of dendrites in information processing is now well established, the exact mechanisms governing the plasticity and pathology of dendritic excitability are still not fully understood. In the first part of this chapter we have reviewed the distribution and function of the major voltage-gated ion channels underlying active dendritic mechanisms. Dendritic voltage-gated ion channels are critical regulators of neuronal activity and plasticity and their malfunction can have important consequences for a wide range of brain disorders. In the second part we have discussed the basic mechanisms of dendritic computation together with changes in dendritic excitability induced through somatic firing activity, local membrane depolarisations, synaptic potentiation, sensory processing and learning. The field of neuronal plasticity research is rapidly expanding providing increasing evidence for the importance of intrinsic excitability in metaplasticity, learning and memory allocation. However, the ultimate proof of whether or not the intrinsic plasticity serves as part of the memory engram itself still needs to be provided. In the final part, we have discussed examples of what is currently known about the dendritic channelopathies underlying neurodevelopmental, neuropsychiatric, neurodegenerative and acquired brain disorders. Taken together, increasing evidence points to the fact that the normal functioning of voltage-gated ion channels is critical for a healthy functioning of the brain. A rise in the number of studies focusing on ameliorating alterations in ion channel function in CNS disorders supports the notion that voltage-gated ion channels are important targets for biomedical research. We hope that our discussion of 'dendritic plasticity' will provide a renewed impetus to studies of this phenomenon in both normal physiology and disease.

References

Abraham WC, Bear MF (1996) Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci.* 19:126–130.

Abraham WC, Gustafsson B, Wigström H (1987) Long-term potentiation involves enhanced synaptic excitation relative to synaptic inhibition in guinea-pig hippocampus. *J. Physiol.* 394:367–380.

Adams JP, Roberson ED, English JD, Selcher JC, Sweatt JD (2000) MAPK regulation of gene expression in the central nervous system. *Acta Neurobiol Exp* (Wars) 60:377–394.

Adelman J, Bond C, Pessia M, Maylie J (1995) Episodic ataxia results from voltage-dependent potassium channels with altered functions. *Neuron* 15:1449–1454.

Aizenman CD, Linden DJ (1999) Regulation of the rebound depolarization and spontaneous firing patterns of deep nuclear neurons in slices of rat cerebellum. *J. Neurophysiol.* 82:1697–1709.

Akhondzadeh S, Mojtahedzadeh V, Mirsepassi G, Moin M, Amini-Nooshabadi H, Kamalipour A (2002) Diazoxide in the treatment of schizophrenia: novel application of potassium channel openers in the treatment of schizophrenia. *J. Clin. Pharm. Ther.* 27:453–459.

Almog M, Korngreen A (2009) Characterization of voltage-gated Ca(2+) conductances in layer 5 neocortical pyramidal neurons from rats. *PLoS ONE* 4:e4841.

Amitai Y, Friedman A, Connors BW, Gutnick MJ (1993) Regenerative activity in apical dendrites of pyramidal cells in neocortex. *Cerebral Cortex* 3:26–38.

Anderson AE, Adams JP, Qian Y, Cook RG, Pfaffinger PJ, Sweatt JD (2000) Kv4.2 phosphorylation by cyclic AMP-dependent protein kinase. *J. Biol. Chem.* 275:5337–5346.

Angelo K, London M, Christensen SR, Häusser M (2007) Local and global effects of I(h) distribution in dendrites of mammalian neurons. *J. Neurosci.* 27:8643–8653.

Arguello P, Gogos J (2012) Genetic and cognitive windows into circuit mechanisms of psychiatric disease. *Trends Neurosci.* 35:3–13.

Ariav G, Polsky A, Schiller J (2003) Submillisecond precision of the input-output transformation function mediated by fast sodium dendritic spikes in basal dendrites of CA1 pyramidal neurons. *J. Neurosci.* 23:7750–7758.

Aronica E, Boer K, Doorn K, Zurolo E, Spliet W, van Rijen P, Baayen J, Gorter J, Jeromin A (2009) Expression and localization of voltage dependent potassium channel Kv4.2 in epilepsy associated focal lesions. *Neurobiol. Dis.* 36:81–95.

Asztely F, Gustafsson B (1994) Dissociation between long-term potentiation and associated changes in field EPSP waveform in the hippocampal CA1 region: an in vitro study in guinea pig brain slices. *Hippocampus* 4:148–156.

Atkinson SE, Williams SR (2009) Postnatal development of dendritic synaptic integration in rat neocortical pyramidal neurons. *J. Neurophysiol.* 102:735–751.

Baio J (2012) Prevalence of autism spectrum disorders-Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008. *MMWR Surveill Summ* 61:1–19.

Barrett C, Tsien R (2008) The Timothy syndrome mutation differentially affects voltage- and calcium-dependent inactivation of CaV1.2 L-type calcium channels. *Proc. Natl. Acad. Sci. USA* 105:2157–2162.

Beck H, Yaari Y (2008) Plasticity of intrinsic neuronal properties in CNS disorders. *Nat. Rev. Neurosci.* 9:357–369.

Becker A, Pitsch J, Sochivko D, Opitz T, Staniek M, Chen C, Campbell K, Schoch S, Yaari Y, Beck H (2008) Transcriptional Upregulation of Cav3.2 Mediates Epileptogenesis in the Pilocarpine Model of Epilepsy. *J. Neurosci.* 28:13341–13353.

Benardo LS, Masukawa LM, Prince DA (1982) Electrophysiology of isolated hippocampal pyramidal dendrites. *J. Neurosci.* 2:1614–1622.

Benarroch EE (2013) HCN channels: function and clinical implications. *Neurology* 80:304–310.

Benhassine N, Berger T (2009) Large-conductance calcium-dependent potassium channels prevent dendritic excitability in neocortical pyramidal neurons. *Pflugers Arch.* 457:1133–1145.

Benito E, Barco A (2010) CREB's control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. *Trends Neurosci.* 33:230–240.

Benvenuto A, Moavero R, Alessandrelli R, Manzi B, Curatolo P (2009) Syndromic autism: causes and pathogenetic pathways. *World J. Pediatr.* 5:169–176.

Bereshpolova Y, Amitai Y, Gusev AG, Stoelzel CR, Swadlow HA (2007) Dendritic backpropagation and the state of the awake neocortex. *J. Neurosci.* 27:9392–9399.

Berger T, Larkum ME, Lüscher HR (2001) High I(h) channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. *J. Neurophysiol.* 85:855–868.

Berger T, Senn W, Lüscher H-R (2003) Hyperpolarization-activated current Ih disconnects somatic and dendritic spike initiation zones in layer V pyramidal neurons. *J. Neurophysiol.* 90:2428–2437.

Bernard C, Anderson A, Becker A, Poolos N, Beck H, Johnston D (2004) Acquired dendritic channelopathy in temporal lobe epilepsy. *Science* 305:532–535.

Berridge MJ (1998) Neuronal calcium signaling. *Neuron* 21:13–26.

Bhat S, Dao D, Terrillion C, Arad M, Smith R, Soldatov N, Gould T (2012) CACNA1C (Cav1.2) in the pathophysiology of psychiatric disease. *Prog. Neurobiol.* 99:1–14.

Bhattacharjee A, Kaczmarek LK (2005) For K+ channels, Na+ is the new Ca2+. *Trends Neurosci.* 28:422–428.

Bi G, Poo M (1998) Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *J. Neurosci.* 18:10464–10472.

Biel M, Wahl-Schott C, Michalakis S, Zong X (2009) Hyperpolarization-activated cation channels: from genes to function. *Physiol. Rev.* 89:847–885.

Bittner KC, Andrasfalvy BK, Magee JC (2012) Ion channel gradients in the apical tuft region of CA1 pyramidal neurons. *PLoS ONE* 7:e46652.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361:31–39.

Bliss TV, Gardner-Medwin AR, Lømo T (1973) Synaptic plasticity in the hippocampal formation. In: Macromolecules and behaviour (Ansell G and Bradley PB, eds) pp 193-203. MacMillan, London, UK.

Bliss TV, Lømo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetised rabbit following stimulation of the perforant path. *J. Physiol.* 232:331–356.

Brager DH, Akhavan A, Johnston D (2012) Impaired dendritic expression and plasticity of h-channels in the fmr1(-/y) mouse model of fragile X syndrome. *Cell Reports* 1:225–233.

Brager DH, Johnston D (2007) Plasticity of intrinsic excitability during long-term depression is mediated through mGluR-dependent changes in I(h) in hippocampal CA1 pyramidal neurons. *J. Neurosci.* 27:13926–13937.

Breton JD, Stuart GJ (2009) Loss of sensory input increases the intrinsic excitability of layer 5 pyramidal neurons in rat barrel cortex. *J. Physiol.* 587:5107–5119.

Brewster AL, Bender RA, Chen Y, Dube C, Eghbal-Ahmadi M, Baram TZ (2002) Developmental febrile seizures modulate hippocampal gene expression of hyperpolarization-activated channels in an isoform- and cell-specific manner. *J. Neurosci.* 22:4591–4599.

Brewster AL, Chen Y, Bender RA, Yeh A, Shigemoto R, Baram TZ (2007) Quantitative analysis and subcellular distribution of mRNA and protein expression of the hyperpolarization-activated cyclic nucleotide-gated channels throughout development in rat hippocampus. *Cereb. Cortex* 17:702–712.

Brown D, Passmore G (2009) Neural KCNQ (Kv7) channels. *Br. J. Pharmacol.* 156:1185–1195.

Burkhalter A, Gonchar Y, Mellor RL, Nerbonne JM (2006) Differential expression of I(A) channel subunits Kv4.2 and Kv4.3 in mouse visual cortical neurons and synapses. *J. Neurosci.* 26:12274–12282.

Busche M, Chen X, Henning H, Reichwald J, Staufenbiel M, Sakmann B, Konnerth A (2012) Critical role of soluble amyloid-β for early hippocampal hyperactivity in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 109:8740–8745.

Buzsáki G (2002) Theta oscillations in the hippocampus. *Neuron* 33:325–340.

Buzsáki G, Penttonen M, Nádasdy Z, Bragin A (1996) Pattern and inhibition-dependent invasion of pyramidal cell dendrites by fast spikes in the hippocampus in vivo. *Proc. Natl. Acad. Sci. USA* 93:9921–9925.

Cai X, Liang C, Muralidharan S, Muralidharan S, Kao J, Tang C, Thompson S (2004) Unique roles of SK and Kv4.2 potassium channels in dendritic integration. *Neuron* 44:351–364.

Cai X, Wei D, Gallagher S, Bagal A, Mei Y, Kao J, Thompson S, Tang C (2007) Hyperexcitability of distal dendrites in hippocampal pyramidal cells after chronic partial deafferentation. *J. Neurosci.* 27:59–68.

Callaway JC, Ross WN (1995) Frequency-dependent propagation of sodium action potentials in dendrites of hippocampal CA1 pyramidal neurons. *J. Neurophysiol.* 74:1395–1403.

Campanac E, Daoudal G, Ankri N, Debanne D (2008) Downregulation of dendritic I(h) in CA1 pyramidal neurons after LTP. *J. Neurosci.* 28:8635–8643.

Carrasquillo Y, Burkhalter A, Nerbonne J (2012) A-type K+ channels encoded by Kv4.2, Kv4.3 and Kv1.4 differentially regulate intrinsic excitability of cortical pyramidal neurons. *J. Physiol.* 590:3877–3890.

Cash S, Yuste R (1998) Input summation by cultured pyramidal neurons is linear and position-independent. *J. Neurosci.* 18:10–15.

Cash S, Yuste R (1999) Linear summation of excitatory inputs by CA1 pyramidal neurons. *Neuron* 22:383–394.

Catterall WA (1995) Structure and function of voltage-gated ion channels. *Annu. Rev. Biochem.* 64:493–531.

Catterall WA (2000) Structure and regulation of voltage-gated Ca2+ channels. *Annu. Rev. Cell Dev. Biol.* 16:521–555.

Catterall WA (2011) Voltage-gated calcium channels. *Cold Spring Harb Perspect Biol* 3:a003947.

Catterall WA, Kalume F, Oakley J (2010) NaV1.1 channels and epilepsy. *J. Physiol.* 588:1849–1859.

Cerda O, Trimmer JS (2010) Analysis and functional implications of phosphorylation of neuronal voltage-gated potassium channels. *Neurosci. Lett.* 486:60–67.

Chan C, Glajch K, Gertler T, Guzman J, Mercer J, Lewis A, Goldberg A, Tkatch T, Shigemoto R, Fleming S, Chetkovich D, Osten P, Kita H, Surmeier D (2011) HCN channelopathy in external globus pallidus neurons in models of Parkinson's disease. *Nat. Neurosci.* 14:85–92.

Chavez-Noriega LE, Bliss TV, Halliwell JV (1989) The EPSP-spike (E-S) component of long-term potentiation in the rat hippocampal slice is modulated by GABAergic but not cholinergic mechanisms. *Neurosci. Lett.* 104:58–64.

Chen C (2005) beta-Amyloid increases dendritic Ca2+ influx by inhibiting the A-type K+ current in hippocampal CA1 pyramidal neurons. *Biochem. Biophys. Res. Commun.* vol. 338 (4) pp. 1913-9.

Chen C, Shen J, Chung N, Min M, Cheng S, Liu I (2012) Retrieval of context-associated memory is dependent on the Ca(v)3.2 T-type calcium channel. *PLoS ONE* vol. 7 (1) pp. e29384.

Chen K, Aradi I, Santhakumar V, Soltesz I (2002) H-channels in epilepsy: new targets for seizure control? *Trends Pharmacol. Sci.* 23:552–557.

Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram T, Soltesz I (2001) Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat. Med.* 7:331–337.

Chen L, Yun S, Seto J, Liu W, Toth M (2003) The fragile X mental retardation protein binds and regulates a novel class of mRNAs containing U rich target sequences. *Neuroscience* 120:1005–1017.

Chen S, Su H, Yue C, Remy S, Royeck M, Sochivko D, Opitz T, Beck H, Yaari Y (2011) An increase in persistent sodium current contributes to intrinsic neuronal bursting after status epilepticus. *J. Neurophysiol.* 105:117–129.

Chen WR, Shepherd GM (1997) Membrane and synaptic properties of mitral cells in slices of rat olfactory bulb. *Brain Research* 745:189–196.

Chen X, Yuan L-L, Zhao C, Birnbaum SG, Frick A, Jung WE, Schwarz TL, Sweatt JD, Johnston D (2006) Deletion of Kv4.2 gene eliminates dendritic A-type K+ current and enhances induction of long-term potentiation in hippocampal CA1 pyramidal neurons. *J. Neurosci.* 26:12143–12151.

Chevaleyre V, Castillo PE (2002) Assessing the role of Ih channels in synaptic transmission and mossy fiber LTP. *Proc. Natl. Acad. Sci. USA* 99:9538–9543.

Chevalier M, Lory P, Mironneau C, Macrez N, Quignard J (2006) T-type CaV3.3 calcium channels produce spontaneous low-threshold action potentials and intracellular calcium oscillations. *Eur. J. Neurosci.* 23:2321–2329.

Chklovskii DB, Mel BW, Svoboda K (2004) Cortical rewiring and information storage. *Nature* 431:782–788.

Christie BR, Eliot LS, Ito K, Miyakawa H, Johnston D (1995) Different Ca2+ channels in soma and dendrites of hippocampal pyramidal neurons mediate spike-induced Ca2+ influx. *J. Neurophysiol.* 73:2553–2557.

Colbert CM, Johnston D (1998) Protein kinase C activation decreases activity-dependent attenuation of dendritic Na+ current in hippocampal CA1 pyramidal neurons. *J. Neurophysiol.* 79:491–495.

Colbert CM, Magee JC, Hoffman DA, Johnston D (1997) Slow recovery from inactivation of Na+ channels underlies the activity-dependent attenuation of dendritic action potentials in hippocampal CA1 pyramidal neurons. *J. Neurosci.* 17:6512–6521.

Cooper EC, Harrington E, Jan YN, Jan LY (2001) M channel KCNQ2 subunits are localized to key sites for control of neuronal network oscillations and synchronization in mouse brain. *J. Neurosci.* 21:9529–9540.

D'Adamo M, Catacuzzeno L, Di Giovanni G, Franciolini F, Pessia M (2013) K(+) channelepsy: progress in the neurobiology of potassium channels and epilepsy. *Front Cell Neurosci.* 7:134.

Dan Y, Poo MM (2006) Spike timing-dependent plasticity: from synapse to perception. *Physiol. Rev.* 86:1033–1048.

Daoudal G, Hanada Y, Debanne D (2002) Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. *Proc. Natl. Acad. Sci. USA* 99:14512–14517.

Darnell J, Jensen K, Jin P, Brown V, Warren S, Darnell R (2001) Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* 107:489–499.

Day M, Carr DB, Ulrich S, Ilijic E, Tkatch T, Surmeier DJ (2005) Dendritic excitability of mouse frontal cortex pyramidal neurons is shaped by the interaction among HCN, Kir2, and Kleak channels. *J. Neurosci.* 25:8776–8787.

Deng W, Aimone JB, Gage FH (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat. Rev. Neurosci.* 11:339–350.

Deng P-Y, Rotman Z, Blundon JA, Cho Y, Cui J, Cavalli V, Zakharenko SS, Klyachko VA (2013) FMRP regulates neurotransmitter release and synaptic information transmission by modulating action potential duration via BK channels. *Neuron* 77:696–711.

Dinan T (1987) Calcium-activated potassium conductance. An alternative to the dopamine hypothesis of neuroleptic action? *Br. J. Psychiatry* 151:455–459.

Disterhoft JF, Oh MM (2006) Learning, aging and intrinsic neuronal plasticity. *Trends Neurosci.* 29:587–599.

Dolmetsch RE, Pajvani U, Fife K, Spotts JM, Greenberg ME (2001) Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. *Science* 294:333–339.

Dong S, Duan Y, Hu Y, Zhao Z (2012) Advances in the pathogenesis of Alzheimer's disease: a re-evaluation of amyloid cascade hypothesis. *Transl Neurodegener* 1:18.

Du W, Bautista J, Yang H, Diez-Sampedro A, You S, Wang L, Kotagal P, Lüders H, Shi J, Cui J, Richerson G, Wang Q (2005) Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. *Nat. Genet.* 37:733–738.

Duflocq A, Le Bras B, Bullier E, Couraud F, Davenne M (2008) Nav1.1 is redominantly expressed in nodes of Ranvier and axon initial segments. *Mol. Cell Neurosci* vol. 39(2) pp. 180-192.

Engbers JDT, Anderson D, Tadayonnejad R, Mehaffey WH, Molineux ML, Turner RW (2011) Distinct roles for I(T) and I(H) in controlling the frequency and timing of rebound spike responses. *J. Physiol.* 589:5391–5413.

Engel AK, Fries P, Singer W (2001) Dynamic predictions: oscillations and synchrony in top-down processing. *Nat. Rev. Neurosci.* 2:704–716.

Estacion M, Gasser A, Dib-Hajj SD, Waxman SG (2010) A sodium channel mutation linked to epilepsy increases ramp and persistent current of Nav1.3 and induces hyperexcitability in hippocampal neurons. *Exp. Neurol* 224:362–368.

Fan Y, Fricker D, Brager DH, Chen X, Lu H-C, Chitwood RA, Johnston D (2005) Activity-dependent decrease of excitability in rat hippocampal neurons through increases in I(h). *Nat. Neurosci.* 8:1542–1551.

Frick A, Johnston D (2005) Plasticity of dendritic excitability. *J. Neurobiol.* 64:100–115.

Frick A, Magee JC, Johnston D (2004) LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. *Nat. Neurosci.* 7:126–135.

Frick A, Magee JC, Koester HJ, Migliore M, Johnston D (2003) Normalization of Ca2+ signals by small oblique dendrites of CA1 pyramidal neurons. *J. Neurosci.* 23:3243–3250.

Gallagher A, Hallahan B (2012) Fragile X-associated disorders: a clinical overview. *J. Neurol.* 259:401–413.

Gandy S (2005) The role of cerebral amyloid beta accumulation in common forms of Alzheimer disease. *J. Clin. Invest.* 115:1121–1129.

Gasparini S, Losonczy A, Chen X, Johnston D, Magee JC (2007) Associative pairing enhances action potential back-propagation in radial oblique branches of CA1 pyramidal neurons. *J. Physiol.* 580:787–800.

Gasparini S, Magee JC (2002) Phosphorylation-dependent differences in the activation properties of distal and proximal dendritic Na+ channels in rat CA1 hippocampal neurons. *J. Physiol.* 541:665–672.

Gentet LJ, Williams SR (2007) Dopamine gates action potential backpropagation in midbrain dopaminergic neurons. *J. Neurosci.* 27:1892–1901.

Goldberg JH, Lacefield CO, Yuste R (2004) Global dendritic calcium spikes in mouse layer 5 low threshold spiking interneurones: implications for control of pyramidal cell bursting. *J. Physiol.* 558:465–478.

Golding NL, Kath WL, Spruston N (2001) Dichotomy of action-potential backpropagation in CA1 pyramidal neuron dendrites. *J. Neurophysiol.* 86:2998–3010.

Golding NL, Spruston N (1998) Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. *Neuron* 21:1189–1200.

Golding NL, Staff NP, Spruston N (2002) Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* 418:326–331.

Gong B, Rhodes KJ, Bekele-Arcuri Z, Trimmer JS (1999) Type I and type II Na(+) channel alpha-subunit polypeptides exhibit distinct spatial and temporal patterning, and association with auxiliary subunits in rat brain. *J. Comp. Neurol.* 412:342–352.

Good T, Murphy R (1996) Effect of beta-amyloid block of the fast-inactivating K+ channel on intracellular Ca2+ and excitability in a modeled neuron. *Proc. Natl. Acad. Sci. USA* 93:15130–15135.

Gosset P, Ait-Ghezala G, Sinet P, Créau N (1999) Isolation and analysis of chromosome 21 genes potentially involved in Down syndrome. *J. Neural Transm Suppl.* 57:197–209.

Grewe BF, Bonnan A, Frick A (2010) Back-Propagation of Physiological Action Potential Output in Dendrites of Slender-Tufted L5A Pyramidal Neurons. *Front Cell Neurosci.* 4:13.

Gross C, Yao X, Pong DL, Jeromin A, Bassell GJ (2011) Fragile X mental retardation protein regulates protein expression and mRNA translation of the potassium channel Kv4.2. *J. Neurosci.* 31:5693–5698.

Gu Y, Barry J, McDougel R, Terman D, Gu C (2012) Alternative splicing regulates kv3.1 polarized targeting to adjust maximal spiking frequency. *J. Biol. Chem.* 287:1755–1769.

Gulledge AT, Kampa BM, Stuart GJ (2005) Synaptic integration in dendritic trees. *J. Neurobiol.* 64:75–90.

Gutman GA, Chandy KG, Grissmer S, Lazdunski M, McKinnon D, Pardo LA, Robertson GA, Rudy B, Sanguinetti MC, Stühmer W, Wang X (2005) International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol. Rev.* 57:473–508.

Hagerman R, Berry-Kravis E, Kaufmann W, Ono M, Tartaglia N, Lachiewicz A, Kronk R, Delahunty C, Hessl D, Visootsak J, Picker J, Gane L, Tranfaglia M (2009) Advances in the treatment of fragile X syndrome. *Pediatrics* 123:378–390.

Hains BC, Saab CY, Waxman SG (2005) Changes in electrophysiological properties and sodium channel Nav1.3 expression in thalamic neurons after spinal cord injury. *Brain* 128:2359–2371.

Hammond C, Bergman H, Brown P (2007) Pathological synchronization in Parkinson's disease: networks, models and treatments. *Trends Neurosci.* 30:357–364.

Hampson D, Gholizadeh S, Pacey L (2012) Pathways to Drug Development for Autism Spectrum Disorders. *Clin. Pharmacol. Ther.* 91:189–200.

Harnett MT, Xu N-L, Magee JC, Williams SR (2013) Potassium Channels Control the Interaction between Active Dendritic Integration Compartments in Layer 5 Cortical Pyramidal Neurons. *Neuron* 79:516–529.

Hasselmo ME, Giocomo LM (2006) Cholinergic modulation of cortical function. *J. Mol. Neurosci.* 30:133–135.

Häusser M, Spruston N, Stuart GJ (2000) Diversity and dynamics of dendritic signaling. *Science* 290:739–744.

Häusser M, Stuart GJ, Racca C, Sakmann B (1995) Axonal initiation and active dendritic propagation of action potentials in substantia nigra neurons. *Neuron* 15:637–647.

Hay E, Hill S, Schürmann F, Markram H, Segev I (2011) Models of neocortical layer 5b pyramidal cells capturing a wide range of dendritic and perisomatic active properties. *PLoS Comput. Biol.* 7:e1002107.

Helmchen F, Svoboda K, Denk W, Tank DW (1999) In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons. *Nat. Neurosci.* 2:989–996.

Hille B (2001) Ionic channels of excitable membranes. Sunderland, MA: Sinauer.

Hoffman DA, Johnston D (1998) Downregulation of transient K+ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. *J. Neurosci.* 18:3521–3528.

Hoffman DA, Johnston D (1999) Neuromodulation of dendritic action potentials. *J. Neurophysiol.* 81:408–411.

Hoffman DA, Magee JC, Colbert CM, Johnston D (1997) K+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature* 387:869–875.

Huang YY, Kandel ER (1994) Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. *Learning & Memory* 1:74–82.

Jaffe DB, Johnston D, Lasser-Ross N, Lisman JE, Miyakawa H, Ross WN (1992) The spread of Na+ spikes determines the pattern of dendritic Ca2+ entry into hippocampal neurons. *Nature* 357:244–246.

Jan LY, Jan YN (2012) Voltage-gated potassium channels and the diversity of electrical signalling. *J. Physiol.* 590:2591–2599.

Jensen CS, Rasmussen HB, Misonou H (2011) Neuronal trafficking of voltage-gated potassium channels. *Mol. Cell Neurosci.* 48:288–297.

Jester JM, Campbell LW, Sejnowski TJ (1995) Associative EPSP--spike potentiation induced by pairing orthodromic and antidromic stimulation in rat hippocampal slices. *J. Physiol.* 484 (Pt 3):689–705.

Johnston D, Christie BR, Frick A, Gray R, Hoffman DA, Schexnayder LK, Watanabe S, Yuan L-L (2003) Active dendrites, potassium channels and synaptic plasticity. *Philos Trans R Soc Lond, B, Biol Sci* 358:667–674.

Johnston D, Magee JC, Colbert CM, Cristie BR (1996) Active properties of neuronal dendrites. *Annu. Rev. Neurosci.* 19:165–186.

Johnston D, Narayanan R (2008) Active dendrites: colorful wings of the mysterious butterflies. *Trends Neurosci.* 31:309–316.

Judy J, Zandi P (2013) A review of potassium channels in bipolar disorder. *Front Genet* 4:105.

Jung HY, Mickus T, Spruston N (1997) Prolonged sodium channel inactivation contributes to dendritic action potential attenuation in hippocampal pyramidal neurons. *J. Neurosci.* 17:6639–6646.

Jung S, Jones T, Lugo J, Sheerin A, Miller J, D'Ambrosio R, Anderson A, Poolos N (2007) Progressive dendritic HCN channelopathy during epileptogenesis in the rat pilocarpine model of epilepsy. *J. Neurosci.* 27:13012–13021.

Jung S, Warner L, Pitsch J, Becker A, Poolos N (2011) Rapid loss of dendritic HCN channel expression in hippocampal pyramidal neurons following status epilepticus. *J. Neurosci.* 31:14291–14295.

Kampa BM, Stuart GJ (2006) Calcium spikes in basal dendrites of layer 5 pyramidal neurons during action potential bursts. *J. Neurosci.* 26:7424–7432.

Katona G, Kaszás A, Turi GF, Hájos N, Tamás G, Vizi ES, Rózsa B (2011) Roller Coaster Scanning reveals spontaneous triggering of dendritic spikes in CA1 interneurons. *Proc. Natl. Acad. Sci. USA* 108:2148–2153.

Kerti K, Lörincz A, Nusser Z (2012) Unique somato-dendritic distribution pattern of Kv4.2 channels on hippocampal CA1 pyramidal cells. *Eur. J. Neurosci.* 35:66–75.

Khosravani H, Altier C, Simms B, Hamming K, Snutch T, Mezeyova J, McRory J, Zamponi G (2004) Gating effects of mutations in the Cav3.2 T-type calcium channel associated with childhood absence epilepsy. *J. Biol. Chem.* 279:9681–9684.

Kim J, Jung S-C, Clemens AM, Petralia RS, Hoffman DA (2007) Regulation of dendritic excitability by activity-dependent trafficking of the A-type K+ channel subunit Kv4.2 in hippocampal neurons. *Neuron* 54:933–947.

Kim J, Wei D, Hoffman D (2005) Kv4 potassium channel subunits control action potential repolarization and frequency-dependent broadening in rat hippocampal CA1 pyramidal neurones. *J. Physiol.* 569:41–57.

Kitamura K, Häusser M (2011) Dendritic calcium signaling triggered by spontaneous and sensory-evoked climbing fiber input to cerebellar Purkinje cells in vivo. *J. Neurosci.* 31:10847–10858.

Koester H, Sakmann B (1998) Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proc. Natl. Acad. Sci. USA* 95:9596–9601.

Koester H, Sakmann B (2000) Calcium dynamics associated with action potentials in single nerve terminals of pyramidal cells in layer 2/3 of the young rat neocortex. *J. Physiol.* 529 Pt 3:625–646.

Kole MHP, Bräuer A, Stuart GJ (2007) Inherited cortical HCN1 channel loss amplifies dendritic calcium electrogenesis and burst firing in a rat absence epilepsy model. *J. Physiol.* 578:507–525.

Kole MHP, Hallermann S, Stuart GJ (2006) Single Ih channels in pyramidal neuron dendrites: properties, distribution, and impact on action potential output. *J. Neurosci.* 26:1677–1687.

Krey J, Dolmetsch R (2007) Molecular mechanisms of autism: a possible role for Ca2+ signaling. *Curr. Opin. Neurobiol.* 17:112–119.

Krzemien DM, Schaller KL, Levinson SR, Caldwell JH (2000) Immunolocalization of sodium channel isoform NaCh6 in the nervous system. *J. Comp. Neurol.* 420:70–83.

Lai HC, Jan LY (2006) The distribution and targeting of neuronal voltage-gated ion channels. *Nat. Rev. Neurosci.* 7:548–562.

Larkum ME, Kaiser KM, Sakmann B (1999a) Calcium electrogenesis in distal apical dendrites of layer 5 pyramidal cells at a critical frequency of back-propagating action potentials. *Proc. Natl. Acad. Sci. USA* 96:14600–14604.

Larkum ME, Nevian T, Sandler M, Polsky A, Schiller J (2009) Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. *Science* 325:756–760.

Larkum ME, Waters J, Sakmann B, Helmchen F (2007) Dendritic spikes in apical dendrites of neocortical layer 2/3 pyramidal neurons. *J. Neurosci.* 27:8999–9008.

Larkum ME, Zhu JJ (2002) Signaling of layer 1 and whisker-evoked Ca2+ and Na+ action potentials in distal and terminal dendrites of rat neocortical pyramidal neurons in vitro and in vivo. *J. Neurosci.* 22:6991–7005.

Larkum ME, Zhu JJ, Sakmann B (1999b) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* 398:338–341.

Laumonnier F, Roger S, Guérin P, Molinari F, M'rad R, Cahard D, Belhadj A, Halayem M, Persico A, Elia M, Romano V, Holbert S, Andres C, Chaabouni H, Colleaux L, Constant J, Le Guennec J, Briault S (2006) Association of a functional deficit of the BKCa channel, a synaptic regulator of neuronal excitability, with autism and mental retardation. *Am. J. Psychiatry* 163:1622–1629.

Laurent G and Borst A (2008) Short stories about small brains: linking biophysics to computation. In: Dendrites (Stuart GJ, Spruston N, Häusser M, eds), pp. 441–464. New York: Oxford University Press.

Lavzin M, Rapoport S, Polsky A, Garion L, Schiller J (2012) Nonlinear dendritic processing determines angular tuning of barrel cortex neurons in vivo. *Nature* 490:397–401.

Lee H, Ge W, Huang W, He Y, Wang G, Rowson-Baldwin A, Smith S, Jan Y, Jan L (2011) Bidirectional Regulation of Dendritic Voltage-Gated Potassium Channels by the Fragile X Mental Retardation Protein. *Neuron* 72:630–642.

Leterrier C, Brachet A, Fache M-P, Dargent B (2010) Voltage-gated sodium channel organization in neurons: protein interactions and trafficking pathways. *Neurosci. Lett.* 486:92–100.

Letzkus JJ, Kampa BM, Stuart GJ (2006) Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. *J. Neurosci.* 26:10420–10429.

Lewis AS, Estep CM, Chetkovich DM (2010) The fast and slow ups and downs of HCN channel regulation. *Channels (Austin)* 4:215–231.

Liao L, Park S, Xu T, Vanderklish P, Yates J (2008) Quantitative proteomic analysis of primary neurons reveals diverse changes in synaptic protein content in fmr1 knockout mice. *Proc. Natl. Acad. Sci. USA* 105:15281–15286.

Lipowsky R, Gillessen T, Alzheimer C (1996) Dendritic Na+ channels amplify EPSPs in hippocampal CA1 pyramidal cells. *J. Neurophysiol.* 76:2181–2191.

Lipscombe D, Andrade A, Allen SE (2013) Alternative splicing: functional diversity among voltage-gated calcium channels and behavioral consequences. *Biochim Biophys Acta* 1828:1522–1529.

Llinás R, Sugimori M (1980) Electrophysiological properties of in vitro Purkinje cell dendrites in mammalian cerebellar slices. *J. Physiol.* 305:197–213.

Lockridge A, Yuan L-L (2011) Spatial learning deficits in mice lacking A-type K(+) channel subunits. *Hippocampus* 21:1152–1156.

London M, Häusser M (2005) Dendritic computation. *Annu. Rev. Neurosci.* 28:503–532.

Lörincz A, Notomi T, Tamás G, Shigemoto R, Nusser Z (2002) Polarized and compartment-dependent distribution of HCN1 in pyramidal cell dendrites. *Nat. Neurosci.* 5:1185–1193.

Lörincz A, Nusser Z (2010) Molecular identity of dendritic voltage-gated sodium channels. *Science* 328:906–909.

Losonczy A, Magee JC (2006) Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron* 50:291–307.

Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* 452:436–441.

Lotharius J, Brundin P (2002) Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. *Nat. Rev. Neurosci.* 3:932–942.

Lugo JN, Barnwell LF, Ren Y, Lee WL, Johnston LD, Kim R, Hrachovy RA, Sweatt JD, Anderson AE (2008) Altered phosphorylation and localization of the A-type channel, Kv4.2 in status epilepticus. *J. Neurochem.* 106:1929–1940.

Lugo JN, Brewster AL, Spencer CM, Anderson AE (2012) Kv4.2 knockout mice have hippocampal-dependent learning and memory deficits. *Learn Mem.* 19:182–189.

Luján R (2010) Organisation of potassium channels on the neuronal surface. *J. Chem. Neuroanat.* 40:1–20.

Luján R, Maylie J, Adelman JP (2009) New sites of action for GIRK and SK channels. *Nat. Rev. Neurosci.* 10:475–480.

Ly C, Melman T, Barth A, Ermentrout G (2011) Phase-resetting curve determines how BK currents affect neuronal firing. *J. Comput. Neurosci.* 30:211–223.

Magee JC (1998) Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *J. Neurosci.* 18:7613–7624.

Magee JC (1999) Dendritic Ih normalizes temporal summation in hippocampal CA1 neurons. *Nat. Neurosci.* 2:508–514.

Magee JC (2000) Dendritic integration of excitatory synaptic input. *Nat. Rev. Neurosci.* 1:181–190.

Magee JC (2008) Dendritic voltage-gated ion channels. In: Dendrites (Stuart GJ, Spruston N, Häusser M, eds), pp: 225-250. New York: Oxford University Press.

Magee JC, Christofi G, Miyakawa H, Christie B, Lasser-Ross N, Johnston D (1995) Subthreshold synaptic activation of voltage-gated Ca2+ channels mediates a localized Ca2+ influx into the dendrites of hippocampal pyramidal neurons. *J. Neurophysiol.* 74:1335–1342.

Magee JC, Christofi G, Miyakawa H, Christie B, Lasser-Ross N, Johnston D (1995) Subthreshold synaptic activation of voltage-gated Ca2+ channels mediates a localized Ca2+ influx into the dendrites of hippocampal pyramidal neurons. *J. Neurophysiol.* 74:1335–1342.

Magee JC, Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* 275:209–213.

Magee JC, Johnston D (2005) Plasticity of dendritic function. *Curr. Opin. Neurobiol.* 15:334–342.

Major G, Larkum ME, Schiller J (2013) Active Properties of Neocortical Pyramidal Neuron Dendrites. *Annu. Rev. Neurosci* 36:1–24.

Makara JK, Losonczy A, Wen Q, Magee JC (2009) Experience-dependent compartmentalized dendritic plasticity in rat hippocampal CA1 pyramidal neurons. *Nat. Neurosci.* 12:1485–1487.

Malenka RC, Nicoll RA (1999) Long-term potentiation--a decade of progress? *Science* 285:1870–1874.

Mantegazza M, Rusconi R, Scalmani P, Avanzini G, Franceschetti S (2010) Epileptogenic ion channel mutations: from bedside to bench and, hopefully, back again. *Epilepsy Res.* 92:1–29.

Marcelin B, Chauvière L, Becker A, Migliore M, Esclapez M, Bernard C (2009) h channel-dependent deficit of theta oscillation resonance and phase shift in temporal lobe epilepsy. *Neurobiol. Dis.* 33:436–447.

Margrie TW, Sakmann B, Urban NN (2001) Action potential propagation in mitral cell lateral dendrites is decremental and controls recurrent and lateral inhibition in the mammalian olfactory bulb. *Proc. Natl. Acad. Sci. USA* 98:319–324.

Marín-Burgin A, Schinder AF (2012) Requirement of adult-born neurons for hippocampus-dependent learning. *Behavioural Brain Research* 227:391–399.

Markram H, Helm P, Sakmann B (1995) Dendritic calcium transients evoked by single back-propagating action potentials in rat neocortical pyramidal neurons. *J. Physiol.* 485 (Pt 1):1–20.

Martina M, Vida I, Jonas P (2000) Distal initiation and active propagation of action potentials in interneuron dendrites. *Science* 287:295–300.

Martina M, Yao GL, Bean BP (2003) Properties and functional role of voltage-dependent potassium channels in dendrites of rat cerebellar Purkinje neurons. *J. Neurosci.* 23:5698–5707.

Mayford M, Siegelbaum SA, Kandel ER (2012) Synapses and memory storage. Cold Spring Harb Perspect Biol 4.

McKay BE, McRory JE, Molineux ML, Hamid J, Snutch TP, Zamponi GW, Turner RW (2006) Ca(V)3 T-type calcium channel isoforms differentially distribute to somatic and dendritic compartments in rat central neurons. *Eur. J. Neurosci.* 24:2581–2594.

Meisler MH, Kearney JA (2005) Sodium channel mutations in epilepsy and other neurological disorders. *J. Clin. Invest.* 115:2010–2017.

Mel BW (1993) Synaptic integration in an excitable dendritic tree. *J. Neurophysiol.* 70:1086–1101.

Meredith R, Holmgren C, Weidum M, Burnashev N, Mansvelder H (2007) Increased threshold for spike-timing-dependent plasticity is caused by unreliable calcium signaling in mice lacking fragile X gene FMR1. *Neuron* 54:627–638.

Miceli F, Soldovieri M, Iannotti F, Barrese V, Ambrosino P, Martire M, Cilio M, Taglialatela M (2011) The Voltage-Sensing Domain of K(v)7.2 Channels as a Molecular Target for Epilepsy-Causing Mutations and Anticonvulsants. *Front Pharmacol.* 2:2.

Mickus T, Jung HY, Spruston N (1999) Properties of slow, cumulative sodium channel inactivation in rat hippocampal CA1 pyramidal neurons. *Biophys. J.* 76:846–860.

Migliore M, Hoffman DA, Magee JC, Johnston D (1999) Role of an A-type K+ conductance in the back-propagation of action potentials in the dendrites of hippocampal pyramidal neurons. *J. Comput. Neurosci.* 7:5–15.

Migliore M, Shepherd GM (2002) Emerging rules for the distributions of active dendritic conductances. *Nat. Rev. Neurosci.* 3:362–370.

Milojkovic BA, Zhou W-L, Antic SD (2007) Voltage and calcium transients in basal dendrites of the rat prefrontal cortex. *J. Physiol.* 585:447–468.

Misonou H, Menegola M, Mohapatra DP, Guy LK, Park K-S, Trimmer JS (2006) Bidirectional activity-dependent regulation of neuronal ion channel phosphorylation. *J. Neurosci.* 26:13505–13514.

Misra S, Kahlig K, George A (2008) Impaired NaV1.2 function and reduced cell surface expression in benign familial neonatal-infantile seizures. *Epilepsia* 49:1535–1545.

Morozov A, Muzzio IA, Bourtchouladze R, Van-Strien N, Lapidus K, Yin D, Winder DG, Adams JP, Sweatt JD, Kandel ER (2003) Rap1 couples cAMP signaling to a distinct pool of p42/44MAPK regulating excitability, synaptic plasticity, learning, and memory. *Neuron* 39:309–325.

Morse T, Carnevale N, Mutalik P, Migliore M, Shepherd G (2010) Abnormal Excitability of Oblique Dendrites Implicated in Early Alzheimer's: A Computational Study. Front Neural Circuits 4.

Mozzachiodi R, Byrne JH (2010) More than synaptic plasticity: role of nonsynaptic plasticity in learning and memory. *Trends Neurosci.* 33:17–26.

Müller C, Beck H, Coulter D, Remy S (2012) Inhibitory Control of Linear and Supralinear Dendritic Excitation in CA1 Pyramidal Neurons. *Neuron* 75:851–864.

Narayanan R, Johnston D (2007) Long-Term Potentiation in Rat Hippocampal Neurons Is Accompanied by Spatially Widespread Changes in Intrinsic Oscillatory Dynamics and Excitability. *Neuron* 56:1061–1075.

Narayanan R, Johnston D (2008) The h channel mediates location dependence and plasticity of intrinsic phase response in rat hippocampal neurons. *J. Neurosci.* 28:5846–5860.

Nevian T, Larkum ME, Polsky A, Schiller J (2007) Properties of basal dendrites of layer 5 pyramidal neurons: a direct patch-clamp recording study. *Nat. Neurosci.* 10:206–214.

Nguyen PV, Kandel ER (1996) A macromolecular synthesis-dependent late phase of long-term potentiation requiring cAMP in the medial perforant pathway of rat hippocampal slices. *J. Neurosci.* 16:3189–3198.

Nieto-Gonzalez JL, Jensen K (2013) BDNF Depresses Excitability of Parvalbumin-Positive Interneurons through an M-Like Current in Rat Dentate Gyrus. *PLoS ONE* 8:e67318.

Nolan MF, Malleret G, Dudman JT, Buhl DL, Santoro B, Gibbs E, Vronskaya S, Buzsáki G, Siegelbaum SA, Kandel ER, Morozov A (2004) A Behavioral Role for Dendritic Integration. *Cell* 119:719–732.

Nolan MF, Malleret G, Lee KH, Gibbs E, Dudman JT, Santoro B, Yin D, Thompson RF, Siegelbaum SA, Kandel ER, Morozov A (2003) The hyperpolarization-activated HCN1 channel is important for motor learning and neuronal integration by cerebellar Purkinje cells. *Cell* 115:551–564.

Notomi T, Shigemoto R (2004) Immunohistochemical localization of Ih channel subunits, HCN1-4, in the rat brain. *J. Comp. Neurol.* 471:241–276.

Nusser Z (2008) Subcellular distribution of neurotransmitter recpetore and voltage-gated ion channels. In Dendrites (Stuart GJ, Spruston N, Häusser M, eds), pp: 155-187. New York: Oxford University Press 155-187.

Nusser Z (2012) Differential subcellular distribution of ion channels and the diversity of neuronal function. *Curr. Opin. Neurobiol.* 22:366–371.

O'Neill J, Senior T, Csicsvari J (2006) Place-selective firing of CA1 pyramidal cells during sharp wave/ripple network patterns in exploratory behavior. *Neuron* 49:143–155.

Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, Takeuchi T, Itohara S, Yanagawa Y, Obata K, Furuichi T, Hensch TK, Yamakawa K (2007) Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. *J. Neurosci.* 27:5903–5914.

Ohira M, Seki N, Nagase T, Suzuki E, Nomura N, Ohara O, Hattori M, Sakaki Y, Eki T, Murakami Y, Saito T, Ichikawa H, Ohki M (1997) Gene identification in 1.6-Mb region of the Down syndrome region on chromosome 21. *Genome Res.* 7:47–58.

Oliva M, Berkovic SF, Petrou S (2012) Sodium channels and the neurobiology of epilepsy. *Epilepsia* 53:1849–1859.

Ozaita A, Martone ME, Ellisman MH, Rudy B (2002) Differential subcellular localization of the two alternatively spliced isoforms of the Kv3.1 potassium channel subunit in brain. *J. Neurophysiol.* 88:394–408.

Pacheco Otalora L, Hernandez E, Arshadmansab M, Francisco S, Willis M, Ermolinsky B, Zarei M, Knaus H, Garrido-Sanabria E (2008) Down-regulation of BK channel expression in the pilocarpine model of temporal lobe epilepsy. *Brain Res.* 1200:116–131.

Pérez-Garci E, Larkum M, Nevian T (2013) Inhibition of dendritic Ca2+ spikes by GABAB receptors in cortical pyramidal neurons is mediated by a direct Gi/o-β-subunit interaction with Cav1 channels. *J. Physiol.* 591:1599–1612.

Perez-Reyes E and Schneider T (1994) Calcium channels: Structure, function, and classification. *Drug Dev. Res.* 33 295-318.

Peters H, Hu H, Pongs O, Storm J, Isbrandt D (2005) Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. *Nat. Neurosci.* 8:51–60.

Pieretti M, Zhang F, Fu Y, Warren S, Oostra B, Caskey C, Nelson D (1991) Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell* 66:817–822.

Plant L, Webster N, Boyle J, Ramsden M, Freir D, Peers C, Pearson H (2006) Amyloid beta peptide as a physiological modulator of neuronal "A"-type K+ current. *Neurobiol. Aging* 27:1673–1683.

Polsky A, Mel BW, Schiller J (2004) Computational subunits in thin dendrites of pyramidal cells. *Nat. Neurosci.* 7:621–627.

Pongs O, Schwarz JR (2010) Ancillary subunits associated with voltage-dependent K+ channels. *Physiol. Rev.* 90:755–796.

Poolos NP, Johnston D (2012) Dendritic ion channelopathy in acquired epilepsy. *Epilepsia* 53 Suppl 9:32–40.

Poolos NP, Migliore M, Johnston D (2002) Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. *Nat. Neurosci.* 5:767–774.

Quirk MC, Blum KI, Wilson MA (2001) Experience-dependent changes in extracellular spike amplitude may reflect regulation of dendritic action potential back-propagation in rat hippocampal pyramidal cells. *J. Neurosci.* 21:240–248.

Ramakers G, Storm J (2002) A postsynaptic transient K(+) current modulated by arachidonic acid regulates synaptic integration and threshold for LTP induction in hippocampal pyramidal cells. *Proc. Natl. Acad. Sci. USA* 99:10144–10149.

Ramsden M, Plant L, Webster N, Vaughan P, Henderson Z, Pearson H (2001) Differential effects of unaggregated and aggregated amyloid beta protein (1-40) on K(+) channel currents in primary cultures of rat cerebellar granule and cortical neurones. *J. Neurochem.* 79:699–712.

Reichold M, Zdebik A, Lieberer E, Rapedius M, Schmidt K, Bandulik S, Sterner C, Tegtmeier I, Penton D, Baukrowitz T, Hulton S, Witzgall R, Ben-Zeev B, Howie A, Kleta R, Bockenhauer D, Warth R (2010) KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. *Proc. Natl. Acad. Sci. USA* 107:14490–14495.

Remy S, Beck H, Yaari Y (2010) Plasticity of voltage-gated ion channels in pyramidal cell dendrites. *Curr. Opin. Neurobiol.* 20:503–509.

Remy S, Csicsvari J, Beck H (2009) Activity-dependent control of neuronal output by local and global dendritic spike attenuation. *Neuron* 61:906–916.

Rhodes P (2006) The properties and implications of NMDA spikes in neocortical pyramidal cells. *J. Neurosci.* 26:6704–6715.

Robinson RB, Siegelbaum SA (2003) Hyperpolarization-activated cation currents: from molecules to physiological function. *Annu. Rev. Physiol.* 65:453–480.

Rosenkranz JA, Frick A, Johnston D (2009) Kinase-dependent modification of dendritic excitability after long-term potentiation. *J. Physiol.* 587:115–125.

Sabatini B, Svoboda K (2000) Analysis of calcium channels in single spines using optical fluctuation analysis. *Nature* 408:589–593.

Scalmani P, Rusconi R, Armatura E, Zara F, Avanzini G, Franceschetti S, Mantegazza M (2006) Effects in neocortical neurons of mutations of the Na(v)1.2 Na+ channel causing benign familial neonatal-infantile seizures. *J. Neurosci.* 26:10100–10109.

Schaller KL, Caldwell JH (2003) Expression and distribution of voltage-gated sodium channels in the cerebellum. *Cerebellum* 2:2–9.

Schiller J, Helmchen F, Sakmann B (1995) Spatial profile of dendritic calcium transients evoked by action potentials in rat neocortical pyramidal neurones. *J. Physiol.* 487 (Pt 3):583–600.

Schiller J, Major G, Koester HJ, Schiller Y (2000) NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature* 404:285–289.

Schiller J, Schiller Y (2001) NMDA receptor-mediated dendritic spikes and coincident signal amplification. *Curr. Opin. Neurobiol.* 11:343–348.

Schiller J, Schiller Y, Stuart GJ, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *J. Physiol.* 505 (Pt 3):605–616.

Schrader L, Ren Y, Cheng F, Bui D, Sweatt J, Anderson A (2009) Kv4.2 is a locus for PKC and ERK/MAPK cross-talk. *Biochem. J.* 417:705–715.

Schreurs BG, Gusev PA, Tomsic D, Alkon DL, Shi T (1998) Intracellular correlates of acquisition and long-term memory of classical conditioning in Purkinje cell dendrites in slices of rabbit cerebellar lobule HVI. *J. Neurosci.* 18:5498–5507.

Schreurs BG, Tomsic D, Gusev PA, Alkon DL (1997) Dendritic excitability microzones and occluded long-term depression after classical conditioning of the rabbit's nictitating membrane response. *J. Neurophysiol.* 77:86–92.

Schulz R, Kirschstein T, Brehme H, Porath K, Mikkat U, Köhling R (2012) Network excitability in a model of chronic temporal lobe epilepsy critically depends on SK channel-mediated AHP currents. *Neurobiol. Dis.* 45:337–347.

Schwindt P, Crill W (1996) Equivalence of amplified current flowing from dendrite to soma measured by alteration of repetitive firing and by voltage clamp in layer 5 pyramidal neurons. *J. Neurophysiol.* 76:3731–3739.

Schwindt PC, Crill WE (1997) Modification of current transmitted from apical dendrite to soma by blockade of voltage- and Ca2+-dependent conductances in rat neocortical pyramidal neurons. *J. Neurophysiol.* 78:187–198.

Shah M, Anderson A, Leung V, Lin X, Johnston D (2004) Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. *Neuron* 44:495–508.

Shah M, Migliore M, Brown D (2011) Differential effects of Kv7 (M-) channels on synaptic integration in distinct subcellular compartments of rat hippocampal pyramidal neurons. *J. Physiol.* 589:6029–6038.

Shin M, Chetkovich DM (2007) Activity-dependent regulation of h channel distribution in hippocampal CA1 pyramidal neurons. *J. Biol. Chem.* 282:33168–33180.

Shruti S, Clem R, Barth A (2008) A seizure-induced gain-of-function in BK channels is associated with elevated firing activity in neocortical pyramidal neurons. *Neurobiol. Dis.* 30:323–330.

Sicca F, Imbrici P, D'Adamo M, Moro F, Bonatti F, Brovedani P, Grottesi A, Guerrini R, Masi G, Santorelli F, Pessia M (2011) Autism with seizures and intellectual disability: possible causative role of gain-of-function of the inwardly-rectifying K+ channel Kir4.1. *Neurobiol. Dis.* 43:239–247.

Sjöström PJ, Häusser M (2006) A cooperative switch determines the sign of synaptic plasticity in distal dendrites of neocortical pyramidal neurons. *Neuron* 51:227–238.

Sjöström PJ, Rancz EA, Roth A, Häusser M (2008) Dendritic excitability and synaptic plasticity. *Physiol. Rev.* 88:769–840.

Small D, Mok S, Bornstein J (2001) Alzheimer's disease and Abeta toxicity: from top to bottom. *Nat. Rev. Neurosci.* 2:595–598.

Smith M, Flodman P, Gargus J, Simon M, Verrell K, Haas R, Reiner G, Naviaux R, Osann K, Spence M, Wallace D (2012) Mitochondrial and ion channel gene alterations in autism. *Biochim. Biophys. Acta* 1817:1796–1802.

Smith R, Sadee W (2011) Synaptic Signaling and Aberrant RNA Splicing in Autism Spectrum Disorders. *Front Syn. Neurosci.* 3:1–8.

Soldovieri M, Castaldo P, Iodice L, Miceli F, Barrese V, Bellini G, Miraglia del Giudice E, Pascotto A, Bonatti S, Annunziato L, Taglialatela M (2006) Decreased subunit stability as a novel mechanism for potassium current impairment by a KCNQ2 C terminus mutation causing benign familial neonatal convulsions. *J. Biol. Chem.* 281:418–428.

Soldovieri M, Cilio M, Miceli F, Bellini G, Miraglia del Giudice E, Castaldo P, Hernandez C, Shapiro M, Pascotto A, Annunziato L, Taglialatela M (2007) Atypical gating of M-type potassium channels conferred by mutations in uncharged residues in the S4 region of KCNQ2 causing benign familial neonatal convulsions. *J. Neurosci.* 27:4919–4928.

Splawski I, Timothy K, Sharpe L, Decher N, Kumar P, Bloise R, Napolitano C, Schwartz P, Joseph R, Condouris K, Tager-Flusberg H, Priori S, Sanguinetti M, Keating M (2004) Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 119:19–31.

Splawski I, Yoo D, Stotz S, Cherry A, Clapham D, Keating M (2006) CACNA1H mutations in autism spectrum disorders. *J. Biol. Chem.* 281:22085–22091.

Spruston N (2008) Pyramidal neurons: dendritic structure and synaptic integration. *Nat. Rev. Neurosci.* 9:206–221.

Spruston N, Schiller Y, Stuart GJ, Sakmann B (1995) Activity-dependent action potential invasion and calcium influx into hippocampal CA1 dendrites. *Science* 268:297–300.

Strumbos J, Brown M, Kronengold J, Polley D, Kaczmarek L (2010) Fragile X mental retardation protein is required for rapid experience-dependent regulation of the potassium channel Kv3.1b. *J. Neurosci.* 30:10263–10271.

Stuart GJ, Häusser M (1994) Initiation and spread of sodium action potentials in cerebellar Purkinje cells. *Neuron* 13:703–712.

Stuart GJ, Häusser M (2001) Dendritic coincidence detection of EPSPs and action potentials. *Nat. Neurosci.* 4:63–71.

Stuart GJ, Sakmann B (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature* 367:69–72.

Stuart GJ, Schiller J, Sakmann B (1997a) Action potential initiation and propagation in rat neocortical pyramidal neurons. *J. Physiol.* 505 (Pt 3):617–632.

Stuart GJ, Spruston N, Sakmann B, Häusser M (1997b) Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends Neurosci.* 20:125–131.

Su H, Sochivko D, Becker A, Chen J, Jiang Y, Yaari Y, Beck H (2002) Upregulation of a T-type Ca2+ channel causes a long-lasting modification of neuronal firing mode after status epilepticus. *J. Neurosci.* 22:3645–3655.

Su T, Cong W, Long Y, Luo A, Sun W, Deng W, Liao W (2008) Altered expression of voltage-gated potassium channel 4.2 and voltage-gated potassium channel 4-interacting protein, and changes in intracellular calcium levels following lithium-pilocarpine-induced status epilepticus. *Neuroscience* 157:566–576.

Sullivan P, Daly M, O'Donovan M (2012) Genetic architectures of psychiatric disorders: the emerging picture and its implications. *Nat. Rev. Genet.* 13:537–551.

Taube JS, Schwartzkroin PA (1988) Mechanisms of long-term potentiation: a current-source density analysis. *J. Neurosci.* 8:1645–1655.

Tsay D, Dudman JT, Siegelbaum SA (2007) HCN1 channels constrain synaptically evoked Ca2+ spikes in distal dendrites of CA1 pyramidal neurons. *Neuron* 56:1076–1089.

Tsubokawa H, Offermanns S, Simon M, Kano M (2000) Calcium-dependent persistent facilitation of spike backpropagation in the CA1 pyramidal neurons. *J. Neurosci.* 20:4878–4884.

Tsubokawa H, Ross WN (1996) IPSPs modulate spike backpropagation and associated [Ca2+]i changes in the dendrites of hippocampal CA1 pyramidal neurons. *J. Neurophysiol.* 76:2896–2906.

Vacher H, Mohapatra DP, Trimmer JS (2008) Localization and targeting of voltage-dependent ion channels in mammalian central neurons. *Physiol. Rev.* 88:1407–1447.

Vacher H, Trimmer JS (2011) Diverse roles for auxiliary subunits in phosphorylation-dependent regulation of mammalian brain voltage-gated potassium channels. *Pflugers Arch.* 462:631–643.

Varga AW, Yuan L-L, Anderson AE, Schrader LA, Wu G-Y, Gatchel JR, Johnston D, Sweatt JD (2004) Calcium-calmodulin-dependent kinase II modulates Kv4.2 channel expression and upregulates neuronal A-type potassium currents. *J. Neurosci.* 24:3643–3654.

Verret L, Mann EO, Hang GB, Barth AMI, Cobos I, Ho K, Devidze N, Masliah E, Kreitzer AC, Mody I, Mucke L, Palop JJ (2012) Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell* 149:708–721.

Vetter P, Roth A, Häusser M (2001) Propagation of action potentials in dendrites depends on dendritic morphology. *J. Neurophysiol.* 85:926–937.

Vossel K, Beagle A, Rabinovici G, Shu H, Lee S, Naasan G, Hegde M, Cornes S, Henry M, Nelson A, Seeley W, Geschwind M, Gorno-Tempini M, Shih T, Kirsch H, Garcia P, Miller B, Mucke L (2013) Seizures and Epileptiform Activity in the Early Stages of Alzheimer Disease. *JAMA* Neurol:1–9.

Wahl-Schott C, Biel M (2009) HCN channels: structure, cellular regulation and physiological function. *Cell Mol. Life Sci.* 66:470–494.

Wang Z, Xu N-L, Wu C-P, Duan S, Poo M-M (2003) Bidirectional changes in spatial dendritic integration accompanying long-term synaptic modifications. *Neuron* 37:463–472.

Watanabe S, Hoffman DA, Migliore M, Johnston D (2002) Dendritic K+ channels contribute to spike-timing dependent long-term potentiation in hippocampal pyramidal neurons. *Proc. Natl. Acad. Sci. USA* 99:8366–8371.

Waters J, Larkum M, Sakmann B, Helmchen F (2003) Supralinear Ca2+ influx into dendritic tufts of layer 2/3 neocortical pyramidal neurons in vitro and in vivo. *J. Neurosci.* 23:8558–8567.

Westenbroek RE, Merrick DK, Catterall WA (1989) Differential subcellular localization of the RI and RII Na+ channel subtypes in central neurons. *Neuron* 3:695–704.

Westenbroek RE, Hell J, Warner C, Dubel S, Snutch T, Catterall WA (1992) Biochemical properties and subcellular distribution of an N-type calcium channel alpha 1 subunit. *Neuron* 9:1099–1115.

Williams SR, Stuart GJ (1999) Mechanisms and consequences of action potential burst firing in rat neocortical pyramidal neurons. *J. Physiol.* 521 Pt 2:467–482.

Williams SR, Stuart GJ (2000a) Backpropagation of physiological spike trains in neocortical pyramidal neurons: implications for temporal coding in dendrites. *J. Neurosci.* 20:8238–8246.

Williams SR, Stuart GJ (2000b) Site Independence of EPSP Time Course Is Mediated by Dendritic Ih in Neocortical Pyramidal Neurons. *J. Neurophysiol.* 83:3177–3182.

Williams SR, Stuart GJ (2003a) Voltage- and site-dependent control of the somatic impact of dendritic IPSPs. *J. Neurosci.* 23:7358–7367.

Williams SR, Stuart GJ (2003b) Role of dendritic synapse location in the control of action potential output. *Trends Neurosci.* 26:147–154.

Wong RK, Prince DA, Basbaum AI (1979) Intradendritic recordings from hippocampal neurons. *Proc. Natl. Acad. Sci. USA* 76:986–990.

Xu C, Qian C, Zhang Z, Wu C, Zhou P, Liang X (1998) Effects of beta-amyloid peptide on transient outward potassium current of acutely dissociated hippocampal neurons in CA1 sector in rats. *Chin. Med. J.* 111:492–495.

Xu J, Kang N, Jiang L, Nedergaard M, Kang J (2005) Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. *J. Neurosci.* 25:1750–1760.

Xu M, Cao R, Xiao R, Zhu M, Gu C (2007) The axon-dendrite targeting of Kv3 (Shaw) channels is determined by a targeting motif that associates with the T1 domain and ankyrin G. *J. Neurosci.* 27:14158–14170.

Xu N-L, Harnett MT, Williams SR, Huber D, O'Connor DH, Svoboda K, Magee JC (2012) Nonlinear dendritic integration of sensory and motor input during an active sensing task. *Nature* 492:247–251.

Xu X, Guo F, Lv X, Feng R, Min D, Ma L, Liu Y, Zhao J, Wang L, Chen T, Shaw C, Hao L, Cai J (2013) Abnormal changes in voltage-gated sodium channels Na(V)1.1, Na(V)1.2, Na(V)1.3, Na(V)1.6 and in calmodulin/calmodulin-dependent protein kinase II, within the brains of spontaneously epileptic rats and tremor rats. *Brain Res. Bull* 96:1–9.

Yaari Y, Yue C, Su H (2007) Recruitment of apical dendritic T-type Ca2+ channels by backpropagating spikes underlies de novo intrinsic bursting in hippocampal epileptogenesis. *J. Physiol.* 580:435–450.

Yuan L-L, Adams JP, Swank M, Sweatt JD, Johnston D (2002) Protein kinase modulation of dendritic K+ channels in hippocampus involves a mitogen-activated protein kinase pathway. *J. Neurosci.* 22:4860–4868.

Yuste R, Tank DW (1996) Dendritic integration in mammalian neurons, a century after Cajal. *Neuron* 16:701–716.

Zamponi GW, Currie KPM (2013) Regulation of Ca(V)2 calcium channels by G protein coupled receptors. *Biochim. Biophys. Acta* 1828:1629–1643.

Zemankovics R, Káli S, Paulsen O, Freund TF, Hájos N (2010) Differences in subthreshold resonance of hippocampal pyramidal cells and interneurons: the role of h-current and passive membrane characteristics. *J. Physiol.* 588:2109–2132.

Zerr P, Adelman J, Maylie J (1998) Episodic ataxia mutations in Kv1.1 alter potassium channel function by dominant negative effects or haploinsufficiency. *J. Neurosci.* 18:2842–2848.

Zhang L, Li X, Zhou R, Xing G (2006) Possible role of potassium channel, big K in etiology of schizophrenia. *Med. Hypotheses* 67:41–43.

Zhang W, Linden DJ (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. *Nat. Rev. Neurosci.* 4:885–900.

Zhou Y, Won J, Karlsson MG, Zhou M, Rogerson T, Balaji J, Neve R, Poirazi P, Silva AJ (2009) CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. *Nat. Neurosci.* 12:1438–1443.

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